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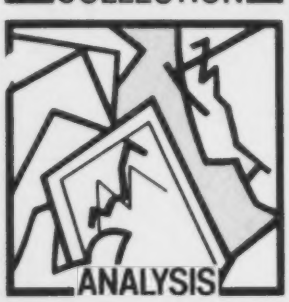

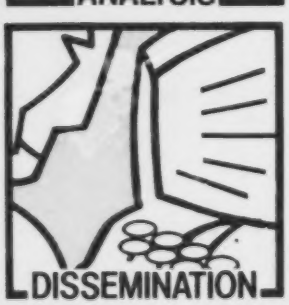

CURRENT CANCER RESEARCH

on

BREAST CANCER: BIOLOGY AND RELATED STUDIES OF ENDOCRINOLOGY AND IMMUNOLOGY

DECEMBER 2, 1980

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • National Institutes of Health •
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Dear Cancer Researcher:

This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 50 different cancer research areas are covered by a separate **SPECIAL LISTING**.

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

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NCI/ICRDB/SL-449
(Updates NCI/ICRDB/SL-349)

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CURRENT CANCER RESEARCH

I. BIOLOGICAL ROLE OF HORMONES AND RELATED ENDOCRINOLOGY

A. MECHANISMS OF HORMONE ACTION AND RELATED METABOLIC STUDIES

1. HORMONE INFLUENCES DURING MAMMARY TUMORIGENESIS

Socher, S. H., Rosen, J. M., Pauley, R. J., Baylor College of Medicine, School of Medicine, Dept. of Cell Biology, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

The goal of this research proposal is to examine the modulation, by steroid and peptide hormones, of gene expression during mouse mammary tumorigenesis. These studies will focus on mouse mammary tumor virus (MMTV) expression in preneoplastic (alveolar nodule outgrowth lines) and neoplastic (derived from nodule lines) mammary tissues in syngeneic high (BALB/cfC3H), moderate (BALB/cVo) and low (BALB/c) mammary tumor incidence strains. Attention will also be directed toward analysis of casein mRNA expression which will be utilized as a biochemical marker of differentiated mammary tissue function. Selective hybridization probes for MMTV RNA and casein mRNA will be used to quantitate RNA and DNA levels in nodules and tumors. Experiments will be carried out primarily in vitro, using an organ culture system which permits precise manipulation of the hormone evironment and provides a culture system in which to measure the hormonal effects on MMTV synthesis and turnover. Cytological studies, including in situ hybridization and immunochemical staining, will be carried out in order to identify the cellular population(s) in preneoplastic and neoplastic mammary lesions that synthesize MMTV and casein and are hormone-responsive. The proposed studies should contribute to our understanding of the interaction of hormonal and viral factors in mammary tumorigenesis.

2. STEROID HORMONES IN BREAST CANCER

Hochberg, R. B., Rosner, W., Hoyte, R. M., Schatz, F., Gasparini, F. J., Roosevelt Hospital, Dept. of Obstetrics & Gynecology, *428 W. 59th St., New York, New York,* 10019, United States of America

Several aspects of steroid biochemistry as they are related to hormonally dependent tumors, especially breast cancer, are to be studied. Our recent identification of a new class of steroids, non-polar lipoidal derivatives in steroidogenic tissue, has been extended and we have isolated a lipoidal derivative of estradiol that has the chemical structure of the non-polar group covalently linked to estradiol. These studies will be performed by radiochemical techniques in conjunction with purification by high pressure liquid chromatography and identification by gas chromatography/mass spectroscopy, as well as other classical methods. Futher experiments will also be carried out to determine the biochemical events involved in the synthesis of this substance as well as its physiological role. The relationship of the lipoidal estrogen to estrogen responsive tissues and the estrogen receptor as well as the endogenous concentration of this substance will be explored. Our success in synthesizing 1 alpha-125I-estradiol, a gamma-emitting derivative of estradiol, which binds with high affinity and specificity to the estrogen receptor will be fully expanded. While the apparent specific activity, greater than 100 Ci/mM, of the iodinated estrogen is now more than adequate for usual receptor studies, attempts are being made to synthesize carrier free tracer (greater than 200 Ci/mM) for increased sensitivity and for use with small clinical samples. The material now available is being used to develop an assay for the detection of estrogen receptor in breast tumors. 131I labelled estradiol is being prepared for in vivo imaging and as a possible palliative agent. In addition, doubly labelled estradiol (125I and 131I) is to be utilized in vitro for experiments on receptor turnover and subcellular localization. Furthermore, 16-alpha-125I-estradiol has been found to bind to antibodies formed against estradiol haptens conjugated at positions C-3, C-6 and C-17 of the steroid nucleus to bovine serum albumin. Sensitive radioimmunoassays for estradiol are being developed. The exchange technique used for the synthesis of the iodinated estrogen is to be used for the synthesis of other radioiodine labelled steroids that bind to progesterone, androgen, and corticoid receptors.

3. HORMONAL PATTERN IN INFLAMMATORY BREAST CARCINOMA

Kiricuta, I., Frenkel, Z., Institutul Oncologic Cluj-Napoca, Hormonal Laboratory, *34-36 Republicii, 3400 Cluj-Napoca, Romania*

OBJECTIVE: Comparative characterization of hormonal pattern in inflammatory breast carcinoma and in breast cancer with slower evolution, in view of a possible use of results in therapy and prognosis of these diseases.

RESEARCH METHODS: The urinary excretion of some steroid hormonal metabolites with spectrophotometric methods both in a group of patients with inflammatory breast carcinoma and in a group with normal evolutive breast cancer. Determinations are carried out for the 24 hr urine (for those in menstrual cycle on the 14th day of cycle). The analysis of total 17-ketosteroids is performed according to a variant of the Zimmermann method and determination of fractionated 17-ketosteroids is carried out by chromatography in thin layer of silica gel. Determination of total estrogens is performed by a variant of the Yayle method. The experimental results are statistically estimated.

PRELIMINARY RESULTS: The data of a limited number of patients show more enhanced hormonal imbalance in inflammatory breast carcinoma than in normal evolutive breast cancer.

4. THE MECHANISM OF ESTROGEN ACTION IN MAMMARY GLANDS

Shyamala, G., Lady Davis Inst. for Medical Research, *3755 Cote St. Catherine Rd., Montreal, Quebec,* H3T 1E2, Canada

OBJECTIVE: The objective of this proposal is to delineate the biological basis for the estrogen-mediated responses in mammary glands.

APPROACH: Progesterone receptors (PgR) are known to be end products of estrogen action in various target tissues for estrogen and we have therefore used PgR to study the estrogenic sensitivity of mammary glands.

PROGRESS: Our studies indicate that the levels of cytoplasmic estrogen receptors (ER) and progesterone receptors (PgR) undergo modulations in normal mammary glands as a function of development and differentiation. ER are present in tissues of all developmental states but increase dramatically during established lactation. PgR are present in mammary glands of virgin and pregnant mice but are totally undetectable during lactation. Furthermore, while estradiol administration to virgin mice results in the augmentation of mammary PgR levels, the same treatment of lactators fails to stimulate the synthesis of mammary PgR. The lack of PgR and the failure of estradiol to augment the levels of PgR during lactation appear specific to mammary glands since the uteri of the same animals have PgR whose levels increase with estradiol administration. At present we are investigating the biological basis for the observed insen-

sitivity of lactating mammary tissue to estradiol with respect to PgR synthesis.

5. TUMOUR PROGRESSION AND BREAST CELL PROGESTIN RESPONSE

Buehring, G. C., Kulski, J. K., University of California, Berkeley Campus, School of Public Health, Dept. of Biomedical & Environmental Health Sciences, *Berkeley, California*, 94720, United States of America

Preliminary studies have shown that the growth of malignant mammary epithelial cells, as opposed to their non-malignant counterparts, is stimulated in vitro by progesterone. This finding suggests that a few already transferred but latent malignant cells in vivo may be selectively stimulated to outgrow normal cells and form a clinically detectable tumor, particularly when the level of progesterone is increased in the peripheral circulation.

The objective of this proposed research is to determine the effect of progesterone and synthetic progestins on the growth, differentiation and interaction of human malignant and non-malignant mammary epithelia using appropriate chemical markers. The effect of the hormones on the transformation of non-malignant to malignant cells will be tested using primary monolayer cultures of different classifications of human mammary epithelium (normal, non-malignant atypical, and malignant). The responses of the cells to hormones will be measured using markers of cell growth (population doubling time and initiated thymidine labelling of DNA), differentiation (production of specific milk products) and interaction (glycosyltransferase activity).

The potential significance of the proposed work is (1) development of chemical monitors for the diagnosis and management of breast cancer, (2) contribution to knowledge of the mechanism of tumor development, (3) possible contributions to better treatment of breast cancer through hormonal manipulation.

6. EFFECT OF LH-RH ANALOGUE AND PROSTAGLANDINS ON PRECANCEROUS MAMMARY HYPERPLASTIC ALVEOLAR NODULES IN MICE

Nagasawa, H., Yanai, R., Ministry of Health & Welfare, Pharmacology Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan*

OBJECTIVE: To evaluate the role of progesterone in mammary tumorigenesis.

APPROACH: A high mammary tumor strain of SHN female mice is given daily i.p. injection of 500, 100 or 10 micrograms of an LH-RH analogue (TAP) or twice daily injections of 50 micrograms of prostaglandin F2 alpha (PGF 2 alpha) or prostaglandin E2 (PGE2) for 20 days. The number of mammary hyperplastic alveolar nodules (HAN) is checked in the wholemount preparations of bilateral third thoracic mammary glands. Plasma levels of prolactin and progesterone are assayed by radioimmunoassay. The age of mice at autopsy is 4 months.

PROGRESS: Administration of 10 micrograms TAP and 50 micrograms PGE2 resulted in the significant increase in the number of HAN associated with elevated plasma progesterone levels and unaltered prolactin level at autopsy.

7. ESTROGEN METABOLISM IN WOMEN WITH BREAST CANCER

Morrel, C., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

8. ESTROGEN/PROGESTIN EFFECTS ON BREAST IN NEONATAL PERIOD

Talamantes, F. J., University of California, Santa Cruz Campus, Graduate School, Dept. of Biology, *Santa Cruz, California*, 95060, United States of America

OBJECTIVE: Determination of the effects of perinatal exposure to exogenous estrogens and progestins, singly and in combination, on mammary gland noduligenesis and tumorigenesis in female mice. The agents whose actions will be examined are diethylstilbestrol (DES), 17 a-hydroxyprogesterone caproate (17-OHPEC), the oral contraceptive Ovral and its components estrogen and progestin, ethinyl estradiol, and norgestrel. These substances are representative of compounds to which human offspring have been frequently exposed in utero (DES, 17-OHPEC, Ovral) and as neonates (Ovral).

9. EFFECTS OF HORMONES AND CHEMICAL CARCINOGENS ON RAT MAMMARY TISSUE IN VITRO

DeOme, K. B., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California*, 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

10. RESPONSE OF EMBRYO MAMMARY GLAND TO ANDROGENIC HORMONES

Kratochwill, K., Osterreichische Akademie der Wissenschaften, Inst. fur Molekularbiologie, Dept. of Biology, *Akademiestrasse 26, 5020 Salzburg, Austria*

OBJECTIVE: Determine the steps in the development of hormone responsiveness in mammary tissue. Specifically, experiments are designed to follow the development of the responsive organ from the beginning of its embryonic life in order to determine when, and under what conditions, it acquires specific responsiveness and to clarify the mechanism of mammary gland regression induced by androgens in the fetus.

11. STUDIES ON HORMONAL MECHANISM(S) REGULATING THE EXPRESSION OF MILK PROTEIN IN RAT MAMMARY GLAND AT DIFFERENT STAGES OF DEVELOPMENT

Qasba, P. K., Nakhasi, H. L., Dart, L., Knox, E., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project is to understand the regulation of the synthesis of milk proteins and their mRNAs in rat mammary gland at different stages of gestation and lactation. The studies include: (a) measurement of the content of alpha-lactalbumin and caseins - 42K, 29K and 25K - along with the levels and activities of their mRNAs in the mammary gland at various stages of gestation and lactation; (b) the rate of synthesis of alpha-LA and caseins in the explant cultures of these glands; (c) identification of the precursors of alpha-LA and casein mRNAs and (d) the influence of hormones, in particular prolactin, on the synthesis of these proteins and their mRNAs in the glands at different stages of gestation.

BIBLIOGRAPHIC REFERENCES: Qasba, P.K., and Nakhasi, H.L.: Abundances of alpha-LA mRNA in 4 day lactating rat mammary gland. Purification of rat alpha-LA mRNA. Proc. Nat. Acad. Sci. 75:4739-4743, 1978.

12. ROLE OF HORMONES IN MAMMARY CARCINOGENESIS (RATS)

Dao, T. L., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

13. ROLE OF HORMONAL FACTORS IN MAMMARY TUMORS

Bogden, A. E., Mason Research Inst. Inc., *21 Harvard St., Worcester, Massachusetts*, 01608, United States of America

OBJECTIVE: Hold virus-inoculated monkeys to be used in biological tests. Holding includes: complete maintenance and general observation; physical exam every six months or when disease of any type is suspected; bleeding for serum every three months (or when specified by the Project Officer), aliquot and storage (no testing); maintenance and health records for each animal; and collection of any biological fluids, tissue collection (via biopsy), or any other manipulation of animals as directed by the Project Officer. Any other test or procedure (RIA hormone determination, histology, pathology, etc.) shall be performed only at the specific direction of the Project Officer on a charge-per-test basis paid for by purchase order.

14. ROLE OF ESTROGEN AND OTHER HORMONES IN BREAST CANCER

Butler, W. B., Kelsey, W., Goran, N., Michigan Cancer Foundation, Dept. of Chemistry, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

Many human breast tumors contain estrogen receptors and many that do are responsive to hormone therapy. The growth of cells from estrogen dependent human and animal tumors in vitro, however, is not estrogen dependent nor, in most cases, estrogen responsive. We propose to test the hypothesis that the role of estrogen in breast cancer is to act not as a mitogen, but rather to induce in the cells properties essential or related to the malignant state, including not only tumor growth but also invasion and metastasis. We will use the human breast cancer cell line MCF-7 as a model system for these studies. MCF-7 requires estrogen in order to form tumors in nude mice but is not dependent on estrogen for growth in culture. We have shown that estrogen at physiological concentrations stimulates the production of plasminogen activator by MCF-7 cells under conditions where the growth rate is unaffected by estrogen. We have clones of MCF-7 which differ in morphology, resistance to the antiestrogen tamoxifen and ability to produce plasminogen activator. We propose to study the effect of hormones, including estrogen, progesterone, insulin and prolactin, on the properties of these clones in vitro. We will measure properties which have been considered as possible markers of transformed cells, including: Colony formation in soft agar, reactivity to concanavalin A, production of plasminogen activator, morphology and growth pattern in collagen-coated sponge cultures. We will also measure resistance to the antiestrogen tamoxifen and the levels of estrogen and progesterone receptors and will test the effect of hormones on these clones in an in vitro assay for tumor cell invasion. The in vitro results will be correlated with the need of the clones for estrogen and their ability to form tumors, invade and metastasize in nude mice. In this way, we will attempt to define those cell properties related to tumor formation by breast cancer cells and the role of hormones, especially estrogen, in the process.

BIBLIOGRAPHIC REFERENCES: Kelsey, W.H., Goran, N. and Butler, W.B. Estrogen Receptor Level Throughout the Cell Cycle of a Human Breast Cancer Cell Line (MCF-7). J. Cell Biol., 79: 12a (abstract No. CC33), (1978). Butler, W.B., Kirkland, W.L. and Jorgensen, T.L. Steroid Stimulation of Fibrinolytic Acitivity in a Human Breast Cancer Cell Line (MCF-7) In: 11th Meeting on Mammary Cancer in Experimental Animals and Man (Brennan, M.J., McGrath, C.M. and Rich, M.A., eds.), 89 (Academic Press, New York), (1979).

15. HORMONES, CELLULAR BIOSYNTHESIS AND MAMMARY CARCINOGENESIS

Banerjee, M. R., University of Nebraska, Lincoln Campus, School of Arts & Sciences, Dept. of Life Sciences, *Lincoln, Nebraska*, 68508, United States of America

Studies will involve assessment of labeled mRNA synthesized in isolated nuclei from glands cultivated in controlled hormonal environment. Hybridization of (3H)RNA synthesized in isolated nuclei will be done under DNA access conditions. Synthesis of the double-stranded form of the cDNAcsn and bacterial cloning of the dscDNAcsn are under way.

BIBLIOGRAPHIC REFERENCES: Senkiti Sakai and Mihir R. Banerjee (1979) Glucocorticoid modulation of prolactin receptors on mammary cells of lactating mice. Biochem. Biophys. Acta 582: 79-88. William J. Bodell and Mihir R. Banerjee (1979) The influence of chromatin structure on the distribution of DNA repair synthesis studied by nuclease digestion. Nucleic Acids Res. 6: 359-370.

16. PROLACTIN STUDIES

Frantz, A. G., Zimmerman, E. A., Wardlaw, S., Ragavan, V., Columbia University, College of Physicians & Surgeons, Dept. of Medicine, *630 W. 168th St., New York, New York*, 10032, United States of America

These studies propose a continuing investigation of prolactin, its characterization and measurement in human beings, the methods by which its secretion is controlled, and its physiological role in normal and diseased states, including pituitary tumors and cancer of the breast. The principal means of investigation will be radioimmunoassay, supplemented by bioassay and radioreceptor assay. Also to be studied are other anterior pituitary hormones, particularly growth hormone, as well as the posterior pituitary peptides vasopressin, oxytocin, and the neurophysins. The peptides beta-endorphin and beta-lipotropin are also being studied.

17. PITUITARY AND THYROID FUNCTION IN BREAST CANCER

Rose, D. P., Davis, T. E., Ruzicka, F. J., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology, *307 N. Charles St., Madison, Wisconsin*, 53706, United States of America

The objective of this project is to define the roles played by thyroid hormones and prolactin in the etiology and biology of breast cancer. This goal will be met by studies of nuclear triiodothyronine, cell membrane prolactin, and cytoplasmic estrogen receptors, and mitochondrial alpha-glycerophosphate dehydrogenase in experimental rat mammary carcinomas induced by N-nitrosomethylurea, and human breast cancer tissues. The influence of thyroid function and circulating prolactin levels on the receptor proteins and the thyroid hormone-inducible mitochondrial enzyme will be studied. Clinical investigations will be concerned with the influence of previous drug administration on tumor receptor levels, particular attention being paid to those known to alter plasma prolactin and estrogen levels.

BIBLIOGRAPHIC REFERENCES: Rose, D.P. and Davis, T.E. Plasma triiodothyronine concentrations in breast cancer. Cancer, 43:1434-1438, 1979. Rose, D.P., Pruitt, B., Stauber, P., Erturk, E. and Bryan, G.T. Influence of dosage schedule on the biological characteristics of N-nitrosomethylurea-induced rat mammary tumors. Cancer Res., 40: 235-239, 1979.

18. HORMONAL INTERACTION IN MAMMARY CARCINOMA

Leung, B. S., Madhok, T., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Obstetrics & Gynecology, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The main purpose of this investigation is to study the effects of prolactin, estrogen and progesterone upon tumor growth and biochemical events. Mammary cancer induced by dimethylbenzanthracene (DMBA) and established cell lines of human or animal origin will be utilized for these studies. Once the effects of a single hormone are known, the interaction of these three hormones will be studied in order to elucidate the mechanism of hormonal action and interaction. In particular, the hormonal effects on the cellular concentrations of receptors, their translocation and nuclear events in gene regulation will be investigated.

This investigation is of paramount importance not only because it helps to elucidate the fundamental principles governing the mechanism of hormonal action and interaction in normal and neoplastic tissues but also because it will provide pertinent information on hormonal dependence and independence in mammary cancers. Practical applications for prognostication, early detection and treatment of this disease are implicated.

19. ROLE OF PROLACTIN IN MOUSE MAMMARY TUMORIGENESIS

Sinha, Y. N., Lewis, U. J., VanderLaan, W. P., Scripps Clinic & Research Foundation, Diabetes & Endocrinology Division, *476 Prospect St., La Jolla, California*, 92037, United States of America

The objective of this proposal is to investigate how prolactin influences the natural development of mammary tumors in mice. We have found that several identifiable variants of mouse prolactin are present in the pituitary glands and sera of mice. We intend to study the significance of these variants in breast cancer development.

The charge isohormones of prolactin in the pituitary gland and size and immunologic variants in the serum will be characterized by electrophoresis, chromatography, radioimmunoassay, bioassay, and receptor assay methods.

Attempts will be made to devise methods of quantitation (radioimmunoassay) for the so far undetectable forms of prolactin in mouse blood.

The interrelationships between the several identifiable forms of mouse prolactin and the genetic, viral, nutritional, and chemical factors important in mouse mammary tumorigenesis will be determined.

BIBLIOGRAPHIC REFERENCES: Sinha, Y.N., Wickes, M.A., Salocks, C.B., and VanderLaan, W.P. Gonadal regulation of prolactin and growth hormone secretion in the mouse. Biol.

Reprod. 21:473-481, 1979. Sinha, Y.N., and Baxter, S.R. Identification of a nonimmunoreactive but highly bioactive form of prolactin in the mouse pituitary by gel electrophoresis. Biochem. Biophys. Res. Commun. 86:325-330, 1979.

20. MECHANISM OF HORMONE ENTRY INTO BREAST CYST FLUID

Bradlow, H. L., Rockefeller University, University Hospital, *66th & York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

21. STEROID AND POLYCYCLIC AROMATIC HYDROCARBON MECHANISMS IN MAMMARY TUMORIGENESIS AND LEUKEMIA

Okey, A. B., University of Windsor, Dept. of Biology, *Windsor, Ontario*, N9B 3P4, Canada

OBJECTIVES: To determine the mechanisms by which steroids and polycyclic aromatic hydrocarbons interact in induction of mammary tumors; to determine the role of microsomal enzyme systems in activation and detoxification of aromatic hydrocarbons.

APPROACH: The effect of parent hydrocarbons and their metabolites on binding of estradiol to estrogen receptor in mammary cytosol is being assessed. The activity of aryl hydrocarbon hydroxylase in microsomes from mammary tissue during different states of susceptibility is being measured.

PROGRESS: Aryl hydrocarbon hydroxylase activity is detectable in the mammary tissue of Sprague-Dawley rats as young as 22 days of age. Basal enzyme activity increases about the time of puberty, then decreases before 50 days of age and remains low throughout ages up to 444 days. Pretreatment of rats with beta-naphthoflavone (BNF) induces a 6-70 fold increase in mammary AHH activity. Inducibility of AHH activity by BNF is greatest in older rats (which have a low sensitivity to tumor induction by polycyclic aromatic hydrocarbons). The inducibility of AHH activity by BNF rises dramatically in mammary tissue during late pregnancy and early lactation.

BIBLIOGRAPHIC REFERENCES: Fysh, J.M. and A.B. Okey, Biochem. Pharmacol. 27:2968-2972 (1978). Fysh, J.M. and A.B. Okey, Canad. J. Physiol. Pharmacol. 57:112-117 (1979).

22. STUDY OF SOME RELATIONSHIPS BETWEEN ESTROGEN METABOLITES AND BREAST CANCER

Cohen, S. L., University of Toronto, Dept. of Obstetrics & Gynecology, *92 College St., Toronto, Ontario*, M5S 1B2, Canada

OBJECTIVES: a) To determine a possible relationship between breast cancer and the occurrrence of 2-hydroxy (catechol)-estradiol in the urine. Preliminary observations tend to indicate such a relationship. b) To determine the nature of the estrogen bound to the nuclear receptors of breast cancer tissue. Preliminary observations indicate that it is either estriol or 2-hydroxy-estradiol (our conditions of assay did not exclude either of these).

APPROACH: a) To carry out our 'profile' assay technique (Cohen et al., Steroids 32, 279, 1978) on urines from patients with breast cancer and a wide variety of controls: normal cycling women; post-menopausal, less than 5 years, 5-10 years, and more than 10 years; women of all ages being subjected to other surgeries. b) To have the catechol estrogens assayed by the specific mass-spectrometric technique of Rosenberg (Hamilton). c) To assay the catechol estrogens by radioimmunoassay techniques (3 different groups of workers have described such a technique).

PROGRESS: a) We have assayed 15 urines (every other day) from the complete 28-30 day cycles of 3 young women. The assays are presently being checked for me by Dr. Diemunn of the Mass. Inst. of Tech. in Boston, by mass-spectrometry. b) An organic chemist (Pauline Zvirsky) is well into the preparation of large quantities of the 3 catechol estrogens for use in the preparation of antibodies. c) I am about to hire someone for the tissue work. Since tissue estrogen assays involve assays which are in the 10 to the minus 6th to the 10 to the minus 9th power of those in urine, we do not like to do both assays in the same room. So we have arranged to have the tissue assay work conducted in Dr. Mobb's laboratory. The NCI Grant does not permit payment of a technician for more than 1/2 time. So I am hiring someone for 1/2 year on the current grant and 1/2 year on next year's grant. She (he) will thus be able to work full time on the project.

23. CHARACTERIZATION OF PLASMINOGEN ACTIVATION ACTIVITY IN HUMAN BREAST TUMOR CYTOSOLS

Sutherland, D. J., Sunnybrook Hospital Medical Centre, *2075 Bayview Ave., Toronto, Ontario*, M4M 3M5, Canada

OBJECTIVE: Plasminogen-activating activities have been demonstrated in human tumors. This study seeks to characterize these activities in human breast tumors.

APPROACH: Human breast tumors being analyzed for steroid receptor proteins will serve as the sources of material. Studies of both cytosol and particulate fractions will be made using recognized separation procedures to characterize the types and molecular characteristics of the plasminogen-activating activities present in these tumors.

PROGRESS: Plasminogen-activating activities have been demonstrated in breast tumors and have been shown to be weakly correlated with steroid receptor activity. Preliminary studies indicate that further characterization of these activities is possible and suggest that characterization of a tumor's plasminogen-activating activity may provide specific markers for tumor-related functions.

24. HORMONES AND BREAST CANCER PROSTAGLANDIN BIOSYNTHESIS

Minton, J. P., Kibbey, W. E., Foecking, M. K., Ohio State University, Columbus Campus, School of Medicine, Dept. of Surgery, *370 W. 9th Ave., Columbus, Ohio*, 43212, United States of America

The hypothesis is proposed that prostaglandin biosynthesis by breast cancers is stimulated by prolactin in tumors which are hormonally sensitive. This is based on evidence that prolactin stimulates prostaglandin production in normal murine mammary tissue. This hypothesis predicts the following experimental observations: 1) Prostaglandin concentrations should be higher in hormonally sensitive tumors; 2) prolactin should stimulate prostaglandin production in hormonally sensitive tumors; 3) estrogens should induce prostaglandin synthetase in hormonally sensitive breast tumors; or 4) estrogens should increase the sensitivity of the prostaglandin synthetase system to prolactin in hormonally sensitive tumors. We plan to test these predictions using radioimmunoassay measurements of tissue prostaglandin E2, prostaglandin synthetase and phospholipase A2 activity in human breast tumors which are classified as hormonally sensitive by the presence of estrogen receptors and in DMBA induced rat mammary tumors which have been exposed to altered (elevated and/or lowered) levels of estrogen and/or prolactin. These latter manipulations will be accomplished by injection of estrogen, ovariectomy and injection of prolactin or administration of prolactin elevating or prolactin release inhibiting drugs.

25. METABOLISM OF ESTROGENS AND ANDROGENS BY BREAST CANCER CELLS

MacIndoe, J. H., Sherman, B. M., Granner, D. K., Ginsberg, B. H., University of Iowa, School of Medicine, Dept. of Internal Medicine, *Jessup Hall, Iowa City, Iowa*, 52240, United States of America

Continuously cultured MCF-7 human breast cancer cells have been recently reported to be responsive to both androgens and estrogens. Studies in this laboratory have confirmed the presence of several enzymes in this cell line that allow the intracellular synthesis of biologically active androgens and estrogens from extracellular precursor steroids. The objectives of this proposal are to 1) identify the spectrum of steroids capable of being transformed by this cell line to active sex steroids and 2) determine whether or not intracellular sex steroid formation can maintain hormone-dependent events in this cell line. Studies include the determination of 5-alpha-reductase, 3-hydroxysteroid dehydrogenase, aromatase, and 17-beta-hydroxysteroid dehydrogenase activities in both whole cultures and cell free MCF-7 systems under a variety of substrate conditions. Studies will also involve examining the effects of various single and combined steroid treatments upon cell protein, steroid enzyme, and DNA contents, as well as alterations in cellular progesterone, estrogen, and androgen receptor levels.

Several studies report that some human breast tumors are capable of forming androgens and/or estrogens from extracellu-

lar precursor. However, investigations into the relative importance of these phenomena in regulating sex hormone dependent cell growth have been hampered by the lack of a suitable model. Our studies now indicate that MCF-7 cells should provide such a system. We anticipate that the results of this study will provide additional insights into the hormonal regulation of human breast cancers, which may eventually lead to improved cancer therapies.

BIBLIOGRAPHIC REFERENCES: MacIndoe, John H., Estradiol Formation from Testosterone by Continuously Cultured Human Breast Cancer Cells, J. Clin. Endocrinol. Metab. 49:272, 1979.

26. MECHANISMS OF HORMONE DEPENDENCE OF HUMAN MALIGNANCY

Lippman, M., Monaco, M. E., Hendler, F., Allegra, J. C., Strobl, J., Chong, M. T., Thompson, E. B., Loriaux, L., Eil, C., Aitken, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Medical Breast Cancer Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings. A. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous disease, melanoma, colon carcinoma, ovarian cancer and male breast cancer. B. Assays for specific gene products (alpha-lactalbumin, casein, fatty acid, synthetase, thymidine kinase) have been developed and we are studying the effects of steroid hormones, insulin and vasopressin on the activities and synthesis of these proteins.

BIBLIOGRAPHIC REFERENCES: Engel, L.W., Young, N.A., Tralka, T.S., Lippman, M.E., O'Brien, S., and Joyce, M.J.: Breast carcinoma cells in continuous culture: Establishment and characterization of three new cell lines. Cancer Research 38: 3352-3364, 1978. Allegra, J.C. and Lippman, M.E.: Growth of a human breast cancer cell line in serum free hormone supplemented medium. Cancer Res. 38: 3823-3829, 1979.

27. HORMONES AND BREAST TISSUE INTERACTIONS

Green, M. R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Protein Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The purpose of this project is to contribute to the understanding of hormone action in metabolic processes in normal breast tissue under various conditions and to understand why hyperplastic or neoplastic breast tissue under the same conditions fails to respond to hormones or responds in an aberrant way. Among the topics studied are the response of mammary tissue in culture to hormones in terms of nucleic acid, protein, phosphoprotein, sialoglycoprotein, glycoprotein and glycosaminoglycan synthesis. The kinds of proteins made by mammary tissue in various stages of differentiation and by neoplastic mammary tissue are being analyzed. Analysis of human milk and formalin-fixed human breast tissue is in progress.

BIBLIOGRAPHIC REFERENCES: Green, M. R.: Simultaneous differential staining of phosphoproteins, sialoglycoproteins, hyaluronic acid, sulfated glycosaminoglycans, proteins and nucleic acids in human breast tissue with a cationic carbocyanine dye. J. Nat. Cancer Inst. 61: 951-960, 1978. Green, M.R., and Pastewka, J.V.: Lactoferrin is a marker for prolactin response in mouse mammary explants. Endocrinology 103: 1510-1513, 1978.

28. STEROID UPTAKE IN HORMONALLY DEPENDENT CANCERS

Barrows, G. H., Wittliff, J. L., Gray, L. A., Spatola, A. F., University of Louisville, School of Medicine, Dept. of Pathology, *101 W. Chestnut, Louisville, Kentucky,* 40202, United States of America

During the first year of the project over 30 new steroid derivatives have been synthesized and labeled with fluorescent agents. In a preliminary trial, strong correlation of in vitro and in vivo uptake properties of these compounds and cell free receptor assays was obtained. Initial structural characterization of the fluorescent steroids has been completed and definitive structural analysis is under way. Initial examination of histochemical methods for detecting steroid receptors reported by others has been completed. During the second year of the project further structural verification of the fluorescent steroids and careful histological, biochemical and clinical correlation of the methods are planned.

BIBLIOGRAPHIC REFERENCES: Barrows, G.H., Stroupe, S.D. and Riehm, J.D. Ethyl succinamide bridged fluorescein 17-beta estradiol as a marker of estrogen dependence. Submitted American Journal of Clinical Pathology (In Press - Accepted June 27, 1979).

29. PROLACTIN SECRETION IN BREAST CANCER (HUMAN)

Malarkey, W. B., Ohio State University, Columbus Campus, School of Medicine, Dept. of Medicine, *370 W. 9th Ave., Columbus, Ohio,* 43212, United States of America

This is part of a broader project. A summary of this subproject is not available.

30. MECHANISM(S) OF STEROID ACTION IN NORMAL MAMMARY GLAND AND IN 7,12-DIMETHYLBENZANTHRACENE (DMBA)-INDUCED MAMMARY TUMORS IN THE RAT

Asselin, J., Kelly, P. A., Belanger, A., Le Centre Hospitalier de l'Universite Laval, Dept. of Molecular Endocrinology, *2705, boul. Laurier, Quebec G1V 4G2, P.Q., Canada*

OBJECTIVE: The objective of this project is to further understand the mechanism(s) of steroid action in normal mammary gland and in DMBA-induced mammary tumors and to compare the results between these two tissues.

APPROACH: Binding characteristics for estrogens, glucocorticoids, androgens and progestins will be studied in cytosol and nuclei as well as nuclear transfer of the steroid receptor complexes in normal mammary gland and in DMBA-induced mammary tumors. Moreover, hormonal factors controlling the levels of four classes of steroid receptors will be investigated in detail.

PROGRESS: The detailed characteristics of estrogens (3H) E2, progestins (3H) R5020, glucocorticoids (3H) Dex and androgens (3H) DHT binding have been determined by cytosol prepared from DMBA-induced mammary tumors. Exchange conditons have been determined for (3H) E2 and (3H) R5020 binding in DMBA cytosol. Moreover, since high levels of (3H) E2 and (3H) Dex binding are present in cytosol prepared from mammary gland of lactating rats, binding characteristics of these two steroids have been studied. Concerning the hormonal factors controlling steroid binding levels in cytosol prepared from DMBA-induced mammary tumors, we have clearly shown that following hypophysectomy or castration, (3H) R5020 binding levels are markedly decreased while (3H) Dex binding remains unchanged. Finally, the data obtained already indicate differences of steroid binding properties or levels in cytosol prepared from DMBA tumors and mammary gland of lactating animals.

31. ESTROGEN METABOLISM IN BREAST CANCER (HUMAN)

Zumoff, B., Montefiore Hospital & Medical Center, *111 E. 210th St., Bronx, New York,* 10467, United States of America

This is part of a broader project. A summary of this subproject is not available.

32. ESTROGEN BIOSYNTHESIS IN ADRENAL AND MAMMARY TISSUE OF MICE, SPRAGUE-DAWLEY RATS TREATED WITH 7,12-DMBA AND IN HUMAN MAMMARY CANCER

Drosdowsky, M. A., Edery, M., Sassier, J. C., Universite de Caen, Faculte de Medecine, Laboratory of Biochemistry, *Av. Cote de Nacre, 14032 Caen, France*

MOUSE MODEL: We have shown that estrogen biosynthesis takes place in vitro in adrenals of F1 (C3H x RIII) mice as well as in the C3H strain. It is much lower in the RIII strain and nil in C57BL (Steroids, in preparation). We have also studied the role of an antiprolactin agent (bromotriphenylethylene in the trans form) in the mammary tumor growth of C3HI/If (XVII) mice implanted with an isologous male pituitary under the kidney capsule. We observed an inhibition of mammary tumor growth, a decrease in serum as well as pituitary prolactin

levels and a decreased binding capacity of prolactin in mammary glands.

RAT MODEL: Using DMBA treated rats, we are studying the aromatization of androgens in mammary tumors and the level of endogenous estrogens.

HUMAN: In collaboration with our Cancer Institute (Centre Francois Baclesse) we are following the same protocol as that for the rat model.

APPROACH: Estrogen determination in tumors using an RIA method derived from that of Thibier et al. (J. Steroid Biochem., 6,1433, 1975) appeared to be unsatisfactory. We are currently measuring E2 using a GC/MS method with a multiple ion detector and a deuterated internal standard. Estrogen biosynthesis is performed using conventional methods (chromatographies on paper, column TLC, crystallization to CSA).

33. BIOCHEMICAL STUDIES ON DMBA INDUCED MAMMARY CANCERS (RATS)

Dao, T. L., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. HORMONE REGULATION STUDIES

34. AROMATASE INHIBITORS FOR CONTROL OF BREAST CANCER

Brodie, A. M., Williams, K. I., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Pharmacology & Experimental Therapeutics, *655 W. Baltimore St., Baltimore, Maryland*, 21201, United States of America

We have prepared compounds which inhibit aromatase (estrogen synthetase) in vitro and have found them to be highly effective in causing regression of DMBA-induced, hormone dependent mammary tumors of the rat. Evidence suggests that aromatase inhibitors act in vivo by inhibiting estrogen biosynthesis: measurements of ovarian secretion were significantly reduced by inhibitor treatment compared to controls and peripheral gonadotropin levels are similar in treated and control rats. Also, when exogenous estradiol was given in addition to inhibitor treatment, tumor regression was prevented. In order to develop aromatase inhibitors as useful compounds for breast cancer therapy and other diseases influenced by estrogen, we propose to investigate further the mechanism of action of aromatase inhibitors. We will determine whether the compounds can inhibit estrogen biosynthesis in vivo by their direct infusion into the externalized ovary of the sheep. We will also determine whether peripheral aromatization can be inhibited in the primate with our present inhibitors. We propose to investigate whether any of the properties of the inhibitors in vivo can be attributed to androgenic activities or whether they can compete for steroid hormone receptors. Using radio-labelled aromatase inhibitors, we are studying the extent of metabolism, distribution and clearance rate of the compounds and whether metabolites contribute to the activity of the inhibitors. By utilizing our structure-activity data we expect to synthesize more potent compounds. An important aspect of our program is the use of affinity labelling and other methods to prepare irreversible inhibitors. We will study the effects of combining aromatase inhibitors with other anticancer agents to determine if more complete and permanent regression can be obtained. We also propose to apply aromatase inhibitors to studying the interrelationships of estrogen with other hormones and their effects on steroid hormone plasma and receptor levels.

35. HORMONAL REGULATION OF THE CYCLIC AMP SYSTEM AND ITS RELATION TO GROWTH OF MAMMARY TUMORS

Shafie, S., Cho Chung, Y. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Insulin and cyclic AMP have been linked to the regulation of mammary tumor growth and regression. Approximately 70 percent of primary DMBA-induced mammary tumors regressed when tumor bearing rats were made diabetic after treatment with streptozotocin. Following insulin withdrawal, significant increases were observed in the cyclic AMP levels, cyclic AMP binding and cyclic AMP-dependent protein kinase activities, both in the cytosol and nuclei. The regulation of the cyclic AMP system by insulin and other hormones is being investigated at the levels of the cyclic AMP phosphodiesterases and will be extended to include adenylate cyclase.

BIBLIOGRAPHIC REFERENCES: Shafie, S.M., Cho-Chung, Y.S. and Gullino, P.M.: Cyclic Adenosine 3',5'-monophosphate and protein kinase activity in insulin-dependent and -independent mammary tumors. Cancer Res. 39: 2501-2504, 1979.

36. HORMONAL CONTROLS OF GROWTH AND DIFFERENTIATION IN CULTURED NORMAL AND NEOPLASTIC MAMMARY CELLS

Lewko, W. M., Kidwell, W. R., Wicha, M. S., Liotta, L. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Although in vivo studies of normal and neoplastic breast tissue indicate that both growth and differentiation processes are hormonally influenced, much uncertainty exists regarding whether the response to hormones is mediated via a direct or indirect interaction of hormone(s) with mammary cells. Furthermore, even in those instances where direct responses to hormones have been observed in in vitro culture experiments, it is unknown whether a specified hormone interacts with one or all of the cellular types of which normal and neoplastic tissues are comprised. Our objective is to assess the peptide and steroid hormone receptor levels in the various cell types (epithelial, myoepithelial and stromal) of normal mammary gland and mammary tumors, to correlate receptor levels with biological responses and to explore the possibility that hormonal actions are the result of coordinated interaction of the various cell types of normal and tumor tissue.

37. HORMONAL INHIBITION OF EXPERIMENTAL BREAST CARCINOGENESIS - MECHANISMS OF ACTION

Jabara, A. G., Cutbush, L. R., Parker, K. G., University of Melbourne, School of Veterinary Science, Dept. of Pathology, *Parkville, Victoria 3052, Australia*

OBJECTIVE: To determine how progestational states occurring relatively early in life confer on the breast a partial resistance to cancer induction.

APPROACH: (1) Animals used - Random-bred Sprague-Dawley rats. (2) Pathological techniques - DMBA intragastric instillation and also direct dusting of mammary gland. (3) Histological techniques - Paraffin sections; mammary gland whole mounts; histochemical studies. (4) Biochemical techniques - Radioimmunoassays of plasma and pituitary PRL and GH; mammary epithelial cell isolation and fractionation; assays of hepatic and mammary microsomal enzymes, e.g., aryl hydrocarbon hydroxylase.

38. FLUOROSTEROIDS AS POTENTIAL INHIBITORS OF AROMATASE AND ESTROGEN DEPENDENT MAMMARY TUMORS

Mann, J., Pietrzak, B., University of Reading, Dept. of Chemistry, *Whiteknights, Reading RG6 2AB, England, United Kingdom*

OBJECTIVE: To synthesize some fluoro-analogues of biosynthetic precursors of estrogens, to study their metabolism, and anti-cancer activity, in hope that they will have activity against estrogen-dependent mammary tumors.

APPROACH: The A-ring of readily available steroids, such as androsten-, 17-dione will be modified, usually by the introduction of one or more fluorine atoms. Metabolism studies will be carried out using commercially available microsomes, and biological evaluation will be carried out in collaboration with the Chester Beatty Research Institute, London.

39. MECHANISM OF ANTIHORMONE ACTION IN BREAST CANCER

McGuire, W. L., University of Texas, Health Science Center at San Antonio, South Texas Medical School, Dept. of Medicine, *7703 Floyd Curl Dr., San Antonio, Texas*, 78284, United States of America

Previous studies in this laboratory have focused on the mechanisms of estrogen and prolactin control of breast tumor growth. It is the particular goal of this proposal to investigate the subcellular biochemistry of androgen action in these tumors

in order to understand the mechanism by which androgens cause regression of breast cancer tissue. A great deal is known about the early events of androgen action in prostate and other male target tissues. It is generally agreed that testosterone (T) enters the cell and is rapidly converted to dihydrotestosterone (DHT), which then binds to a specific cytoplasmic receptor protein. The steroid complex enters the nucleus, where it presumably acts to stimulate the biochemical chain of events necessary for growth and function. In the genetically determined testicular feminization syndrome described in mice, rats, and humans, it has been proposed that the failure of target organ development is causally related to a lack of the specific cytoplasmic DHT receptor. It is unlikely that androgen-induced regression in breast cancer is mediated by this receptor because of the very large pharmacologic dose requirement.

We are proposing possible mechanisms by which pharmacological androgen administration might result in tumor regression. These proposals will be tested in DMBA-induced rat mammary carcinomas, relating the tumor growth response after androgen treatment to several properties of pre- and post-treatment tumor biopsy specimens.

40. HORMONAL REGULATION OF GROWTH, DIFFERENTIATION AND TUMORIGENESIS OF HUMAN BREAST TISSUES IN VIVO

Welsch, C. W., Michigan State University, School of Human Medicine, Dept. of Anatomy, *Soil Science Bldg., East Lansing, Michigan*, 48824, United States of America

OBJECTIVES: A. To define the hormonal requirements for growth of human breast tissues in vitro and in vivo: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 2-4 days in a hormone enriched chemically defined culture media. Growth of the explants will be assessed by 3H-thymidine incorporation into DNA and mitotic figure analysis. 2. Slices of normal human breast tissues will be transplanted to and maintained (1-6 months) in hormone treated athymic 'nude' mice. Growth of the grafts will be assessed by 3H-thymidine incorporation into DNA and mitotic figure analysis. B. To define the hormonal requirements for differentiation (lactation) of human breast tissues in vitro and in vivo: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 4 days in a hormone enriched chemically defined culture media. Differentiation of the explants will be assessed by histological analysis and by alpha-lactalbumin radioimmunoassay. 2. Slices of normal human breast tissues will be transplanted to and maintained (1-6 months) in hormone treated athymic 'nude' mice. Differentiation of the grafts will be assessed by histological analysis and by alpha-lactalbumin radioimmunoassay. C. To induce neoplastic transformation of normal human breast tissues in vitro-in vivo by chemical carcinogens: 1. Organ cultures (explants) of normal human breast tissues will be maintained for 2 days in a carcinogen enriched chemically defined culture media. After culture, the explants will be removed and transplanted to and maintained (1-12 months) in hormone treated athymic 'nude' mice. Neoplastic transformation of the transplanted human breast tissues will be assessed by periodic palpation and histological analysis of the grafted tissue.

41. NEUROENDOCRINE CONTROL OF MAMMARY AND PITUITARY TUMORS

Meites, J., Leung, F., Sonntag, W., Fanjul, L. F., Forman, L., Ruiz de Galarreta, C. M., Michigan State University, School of Veterinary Medicine, Dept. of Physiology, *New Administration Bldg., East Lansing, Michigan*, 48823, United States of America

OVERALL OBJECTIVE: To determine the relation of the brain, particularly the hypothalamus, to development and growth of mammary and pituitary tumors, and to utilize this information to inhibit development and growth of mammary and pituitary tumors. Since prolactin and estrogen are the principal hormones involved in development and growth of mammary tumors, and to a large extent of pituitary tumors, and since these hormones are controlled directly (PRL) or indirectly (estrogen) by the hypothalamus, our aim is to experimentally alter hypothalamic function (by placing lesions or implanting hormones and drugs in the hypothalamus, by administration of drugs and hormones systemically and by use of appropriate environmental stimuli) to inhibit or accelerate development and growth of these tumors in mice, rats and dogs. The effects of high and low fat diets, reduced caloric intake and hypothalamic induced obesity on development and growth of mammary tumors, and on serum hormone levels, will be tested. The neuroendocrine mechanisms whereby pregnancy and lactation influence mammary tumor growth will be investigated. The effects of hypothalamic factors, hormones and drugs on hormone secretion and growth of pituitary tumors also will be studied. Old female rats also will be utilized since they develop spontaneous mammary and pituitary tumors, which we believe are related to functional changes in hypothalamic functions in old rats. We hope that some of the knowledge gained from these studies will prove useful in developing treatments for inhibiting the onset and/or growth of mammary and pituitary tumors in human subjects.

BIBLIOGRAPHIC REFERENCES: Advis, J.P., Simpkins, J.W., Bennett, J. and Meites, J. Serotonergic control of prolactin release in male rats. Life Sciences 24:359-366, 1979. Van Vugt, D.A. and Meites, J. Evidence that endogenous opioid peptides participate in control of anterior pituitary function. In: Proc. FASEB Symp. on Relation of Endogenous Opiate Peptides to Secretion of Hormones, Dallas, Texas, April 1979 (in press).

42. MECHANISM OF HORMONAL CONTROL OF MAMMARY CARCINOMA

McGuire, W. L., University of Texas, Health Science Center at San Antonio, South Texas Medical School, Dept. of Medicine, *7703 Floyd Curl Dr., San Antonio, Texas*, 78284, United States of America

This project entails the study of proteins synthesized in human breast cancer cells as influenced by steroid hormones.

BIBLIOGRAPHIC REFERENCES: McGuire, W.L., Horwitz, K.B., Zava, D.T., and Garola, R.E.: Estrogen and progesterone receptors in human breast cancer, In: Steroid Receptors and the Management of Cancer, E.B. Thompson and M. Lippman (eds.), CRC Press, Cleveland, In Press, 1979. Osborne, C.K., and McGuire, W.L.: Breast cancer therapy: Current status of hormone receptors. Western Journal of Medicine, 130: 401-407, 1979.

43. STUDY ON GROWTH AND REGRESSION IN RAT MAMMARY TUMORS

Lee, C., Oyasu, R., Northwestern University, Chicago Campus, School of Medicine, Dept. of Urology, *303 E. Chicago Ave., Chicago, Illinois*, 60611, United States of America

The overall objective of this proposal is to study the biological processes regulating the growth and regression of 7,12-dimethylbenz(a) anthracene (DMBA)-induced mammary tumors in rats. Most DMBA-induced mammary tumors are hormone-dependent and their growth or regression is regulated by the host's endocrine status. This proposal is based on the principle that any tissue growth is a result of a net gain of synthetic processes over tissue degradation. Thus, tissue synthesis and degradation play equally important roles in tumor growth. Understanding these processes could lead to the development of means to control malignant growth. The following subjects are the areas of our interest for the period proposed: (1) Differential and synergistic roles of estrogen and prolactin in protein and DNA synthesis in DMBA-induced mammary tumors. (2) Interrelationships between synthesis and degradative processes in growing and regressing tumors. (3) Whether or not tumor regression involves processes of active synthesis.

BIBLIOGRAPHIC REFERENCES: C. Lee, L. Oliver, E.L. Coe and R. Oyasu. (1979). Lactate dehydrogenase in normal mammary glands and 7,12-dimethylbenz(a)anthracene-induced mammary tumors in Sprague-Dawley rats. Journal of the National Cancer Institute 62:193-199.

44. ROLE OF INSULIN IN HORMONAL CONTROL OF BREAST CANCER

Hilf, R., University of Rochester, School of Medicine & Dentistry, Dept. of Biochemistry, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

In order to gain an understanding of the nature of hormonal dependency and autonomy of breast cancers, a basic understanding of the action of hormones at the cellular and subcellulr level will be required. Two experimental mammary tumors in rodents are being studied. To examine the actions of insulin at the cellular level, we will employ dissociated tumor cells from

hormonally modified animals, and will examine in vitro the characteristics of insulin binding to its receptor, the actions of insulin on glucose and amino acid transport, the utilization of the transported substrates and the effects of estrogen and other hormones on the regulation of insulin binding and its action in these tumors. Short-term tissue culture of tumor cells, which has been initiated successfully, will be used to examine hormonal regulaton of insulin and estrogen binding in vitro, and the effects of these hormones on growth and metabolism of these cells. Since we perceive that insulin demonstrates both direct and permissive actions, the ultimate goal of the studies is to define these actions of insulin and determine how these may be modified by other hormones, and to relate such mechanisms to tumor growth and its control.

BIBLIOGRAPHIC REFERENCES: Effects of Sulfhydryl Environment on Insulin Binding to Mammary Tumor Cells. S.M. Shafie and R. Hilf. Fed. Proc., 38:349, 1979. Modulation of Chemical Carcinogenesis by Hormones. R. Hilf. In: Biochemical Actions of Hormones (G. Litwack, ed.), Vol. VI, pp. 205-245. New York: Academic Press, Inc., 1979, in press.

45. PROLACTIN CELL FUNCTION IN BREAST CANCER

Hymer, W. C., Pennsylvania State University, University Park Campus, School of Science, Microbiology, Cell Biology, Biochemistry & Biophysics, *211 Whitmore Lab., University Park, Pennsylvania*, 16802, United States of America

Pituitary prolactin (PRL) cells from F-344 rats, both normal and mammary tumor bearing, are being studied in vitro. The presence of the 13762 mammary tumor suppresses pituitary PRL and GH function. This suppression is reversible after surgery or chemotherapy.

Addition of rat serum (RS) to cells in culture markedly suppresses PRL release. Evidence to date shows that the inhibitory material is associated with proteins of MW 120,000 and 30,000.

Efforts are underway to develop a serum-free culture system for PRL cells.

Finally, we have developed a method for the testing of dispersed PRL cells in vivo. This involves encapsulation of cells in Amicon hollow fibers prior to their implantation in certain ectopic sites (brain, femur, kidney capsule). After 1-14 days the capsules are removed and studied in vitro for PRL release. Results indicate that placement site markedly affects subsequent secretory function.

BIBLIOGRAPHIC REFERENCES: Wilfinger, W., Davis, J., Augustine, E., and Hymer, W.C., The Effects of Culture Conditions on Prolactin and Growth Hormone Production by Rat Anterior Pituitary Cells. Endocrinology 105:530-536, 1979. Hymer, W.C., and Signorella, A., Feedback Effects of Mammary Gland Tumors on the Host Pituitary Prolactin Cell, in: Hormones and Cancer (W. Leavitt, ed.), Plenum Pres, in press (1980).

46. CONTROL OF NEOPLASTIC GROWTH BY TESTOSTERONE

Bruchovsky, N., Cancer Control Agency of British Columbia, Dept. of Cancer Endocrinology, *2656 Heather St., Vancouver, British Columbia*, V5Z 3J3, Canada

OBJECTIVE: To identify defects of regulation that occur in hormone-sensitive and insensitive tumors of prostate and breast.

APPROACH: Variations in the molecular phenotypes of normal and malignant cells are being defined in order to establish whether anomalous growth patterns can be attributed to one or several of the following related factors: (1) Fluctuations in the net concentration of active androgens in the cell; (2) changes in the distribution of androgens between cytoplasm and the nucleus; (3) interactions between androgens, or androgen receptor complexes, and chromatin; (4) hormone-induced temporal variations in the organization and transcription of chromatin. The animal models to be used in the studies are the rat prostate, the transplantable estrogen-dependent rat mammary and prostate carcinomas isolated by Noble. Within certain limits, principles that evolve from the study of animal models will be used as a basis for parallel studies on hyperplastic and neoplastic conditions of human breast and prostate.

PROGRESS: Radioimmunoassays for dihydrotestosterone and testosterone were developed and the concentrations of these androgens in whole-tissue and nuclei were measured during the cycles of growth, regression and regrowth of tumors. The results accumulated to date indicate that under steady-state conditions dihydrotestosterone is localized exclusively in the nucleus while testosterone is confined almost entirely to the cytoplasm. After castration the concentration of testosterone declines more rapidly than that of dihydrotestosterone. Other as yet incomplete results suggest that tumor regrowth is not accompanied by any elevation of the whole-tissue concentration of either androgen.

Screening of human prostatic tissue for phenotypic markers of hormonal sensitivity was also started. In addition to the aforementioned radioimmunoassays, procedures were developed to measure the concentration of nuclear androgen receptor and the activities of the androgen-metabolizing enzymes, 5 alpha-reductase and 3 alpha(beta)-hydroxysteroid dehydrogenase. The results compiled so far are in keeping with the following conclusions: (1) The concentration of androgen receptor is 1000-2000 molecules/nucleus; (2) the nuclear concentration of unbound androgen is 15- to 30-fold greater; and (3) 5 alpha-reductase activity is 10% of normal in carcinoma and 29.0% of normal in hyperplasia.

47. INTERRELATIONSHIP OF CYCLIC NUCLEOTIDE AND STEROID HORMONE IN GROWTH CONTROL

Cho Chung, Y. S., Bodwin, J. S., Clair, T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A correlation was found between the antagonistic effects of cAMP and estrogen on nuclear protein phosphorylation in vitro and the hormone-dependency of mammary tumor growth in vivo. The antagonistic action between cAMP and estrogen was further revealed on the phosphorylation of the 56,000-dalton cAMP binding protein in the cytoplasm of hormone-dependent tumors, suggesting a close relation between the phosphorylation of cAMP binding protein and phosphorylation of nuclear proteins. These results provide further support for our initial proposal that the growth control of hormone-dependent mammary tumors is dependent on the interaction of cAMP and estrogen.

BIBLIOGRAPHIC REFERENCES: Cho-Chung, Y.S., Bodwin, J.S., and Clair, T.: Cyclic AMP-binding proteins: Inverse relationship with estrogen-receptors in hormone-dependent mammary tumor regression. Eur. J. Biochem. 86: 51-60, 1978. Cho-Chung, Y.S.: Interaction of cyclic AMP and estrogen in tumor growth control. In Sharma, R.K., and Criss, W.E. (Eds.): Endocrine Control in Neoplasia. New York, Raven Press, 1978, pp. 335-346.

48. SUPPRESSION OF ENDOCRINE FUNCTION IN HUMAN BREAST CANCER

Santen, R. J., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Medicine, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

GOAL: To produce suppression of endocrine function with aminoglutethimide (AG), to establish the mechanism of steroid inhibition, and to compare the effects of surgical adrenalectomy, antiestrogen therapy, and aminoglutethimide in breast carcinoma.

WORKSCOPE: Female patients with inoperable recurrent or metastatic breast carcinoma and whose tumors are either estrogen receptor positive or unknown are selected for study. Women are randomized into two separate therapeutic trials: a comparison of medical adrenalectomy (AG plus hydrocortisone) vs. surgical adrenalectomy, and of AG vs. the antiestrogen, tamoxifen. Extensive endocrine studies evaluate the effects of AG on extra-adrenal estrogen production, androgen, progestin, and prolactin secretion, and steroid metabolism.

49. PROLACTIN RECEPTOR REGULATION IN CULTURED MAMMARY TUMORS

Costlow, M. E., St. Jude Childrens Research Hospital, Dept. of Biochemistry, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee*, 38101, United States of America

The objective of the proposed research is to characterize prolactin receptor regulation in hormone-dependent mammary

carcinoma. Using primary cultures of DMBA-induced rat mammary tumors which retain prolactin receptors and prolactin responsiveness, we will investigate (1) the direct effects of insulin, progesterone, androgen and estrogen on the level of prolactin receptor sites, (2) the autoregulation of prolactin receptors, (3) the cellular location of prolactin and prolactin receptor sites following prolactin treatment of cultured tumor cells from autoradiographic and immunocytochemical studies, and (4) any cell-cycle-specific changes in prolactin receptors. We will determine if the activities of guanyl cyclase, ornithine decarboxylase and the uptake of alpha-amino-isobutyric acid are influenced by prolactin and correlated with the number of prolactin receptors and tumor growth. The results of these studies should provide a more complete understanding of the mechanism by which prolactin controls the growth of hormone-dependent mammary tumors.

50. EFFECT OF AGING ON HORMONAL CONTROL OF MAMMARY TUMORS

Hsueh, A. J., Erickson, G. F., University of California, San Diego Campus, School of Medicine, Dept. of Reproductive Medicine, *P.O. Box 109, La Jolla, California,* 92093, United States of America

The overall objective of the project is to delineate the role of reproductive hormones in the induction and development of spontaneous mammary tumors (SMT) in aging female rats. We plan to correlate the changes in reproductive patterns (and reproductive hormones) with the incidence and growth of spontaneous mammary tumors in order to study the hormonal responsiveness of these tumors. We have studied the hormonal responsiveness of a population of aging female rats bearing spontaneous mammary tumors. Following ovariectomy of tumor-bearing rats, the majority of the SMT in these rats exhibited pronounced decrease in tumor size and subsequent regrowth with a lag period of 1 to 2 months, suggesting hormonal dependency of SMT. A second population of 50 aging female rats has also been maintained to study the effect of hormonal ablation therapies. Histological examination indicated that most of SMT studied are adenofibromas exhibiting varying degrees of secretory activity. In search of a tumor marker for monitoring the possible hormonal regulation of tumor growth, we measured alpha-LA (B protein of lactose synthetase) content in the cytosol fraction (10,000 x g of SMT by a specific radioimmunoassay procedure employing staphylococcus A to precipitate antigen-antibody complexes). High concentration of alpha-LA was found in mammary tissues of lactating rats (21.9 plus or minus 5.6 micrograms/mg protein; mean plus or minus S. E., n equals 6), whereas no alpha-LA was detected in uterus, kidney, fat, heart muscle thymus, and ovary, indicating the tissue specificity of alpha-LA production. In contrast, all 12 SMT examined exhibited varying but significant amounts of alpha-LA. Alpha-LA concentration is greater than 0.1% of the total protein in 66% of the SMT, whereas the remaining SMT have less than 1 microgram alpha-LA/mg protein. These results demonstrate the substantial elevation of alpha-LA in SMT of aging female rats and suggest that alpha-LA may serve as a specific marker for monitoring the hormonal regulation of spontaneous mammary tumorigenesis.

51. ISOLATION AND CHARACTERIZATION OF PROLACTIN INHIBITING FACTOR (PIF) AND PROLACTIN RELEASING FACTOR (PRF)

Dupont, A., Asselin, J., Belanger, A., Moore, S., Kelly, P. A., Labrie, F., Le Centre Hospitalier de l'Universite Laval, Dept. of Molecular Endocrinology, *2705, boul. Laurier, Quebec G1V 4G2, P.Q., Canada*

The existence of a prolactin inhibiting factor (PIF) and a prolactin releasing factor (PRF) is suggested by many experimental approaches (pituitary transplantation, pituitary stalk section, lesions of the hypothalamus, electrical stimulation of specific hypothalamic areas and infusion of hypothalamic extracts). Therefore, isolation and knowledge of the structure of these hypothalamic hormones appear to be of prime interest. In fact, the objective remission of breast cancer induced by L-dopa or ergot derivatives suggests a possible use of PIF or PRF antagonists in the therapy of breast cancer. Porcine hypothalami extracted will be subsequently purified by techniques based upon the molecular size of the polypeptides, their partitioning properties and their amphoteric characteristics. An assay using dispersed anterior pituitary cells in monolayer culture will be used to screen the biological inhibiting or stimulating activity of prolactin secretion in each fraction. The fractions active in vitro will be then assayed in in vivo tests specially designed for the hypothalamic hormone. TLC and TLE will be mainly used to determine the purity of the fractions.

After purification, isolation and elucidation of the structures of PIF and PRF, synthesis of the new molecules, and comparison of their biological activity against the natural molecules will be undertaken.

The effect of synthetic PIF and of PRF antagonist upon growth of breast tumor induced by 7,12-dimethylbenzanthracene (DMBA) would then be investigated. Studies of the inactivation and organ distribution of the synthetic peptides will also be performed in view of potential clinical use. The possible role of prolactin in breast cancer and the relatively high frequency of nonpuerperal galactorrhea indicate clearly the importance of gaining knowledge about the nature of the hypothalamic factors regulating PRL secretion. Knowledge of the structure of these substances should permit synthesis of compounds of clinical use.

52. HORMONE INTERACTION IN REGULATION OF MAMMARY CARCINOGENESIS

Venter, B., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

53. ENDOCRINE CONTROL OF INDUCTION AND GROWTH OF EXPERIMENTAL MAMMARY TUMORS

Mouriquand, J. C., Jacrot, M., Laboratoire d'Histologie, *38700 La Tronche, France*

OBJECTIVE: Endocrine control of DMBA induced mammary tumors in rats has been well described. The same approach is applied to mammary tumors induced in female Sprague-Dawley rats by fast neutron irradiations (I4 MeV).

APPROACH: Single or fractionated low doses of fast neutrons are applied, at the onset of puberty, to virgin females under several pituitary gland stimulations. Endocrine control of the tumor bearing animal: estrogen and progesterone receptors of the tumors are compared with those obtained in DMBA induced tumors under similar pituitary stimulation. Two cell lines have been obtained from such tumors and are studied in vitro.

54. REGULATORY RELATIONSHIPS BETWEEN THE ACTIONS OF STEROID HORMONES ON HUMAN BREAST CANCER CELLS IN CULTURE

Bayard, F., Laboratoire d'Endocrinologie Experimentale, *c/o C.H.R. Rangueil BT 3, Toulouse,* 31, France

OBJECTIVE: To characterize the relationships between the actions of different steroid hormones on the same cancer cell.

APPROACH: Breast cancer cells, grown in culture, are selected for their responsiveness to 4 groups of steroid hormones: estrogens, progestins, androgens, and glucocorticoids. The action of one hormone of each group on the receptor level, metabolic enzyme activity of the other three groups, as well as cell growth parameters are studied. At the present time, studies concern mainly relationships between estrogens and progestins.

55. STEROID HORMONE REGULATION OF GROWTH IN MAMMARY TUMOR CELL LINES

Rochefort, H., Vignon, F., Westley, B., Inst. National de la Sante et de la Recherche Medicale, Unite 148, *60 Rue de Navacelles, 34100 Montpellier, France*

OBJECTIVE: To analyze the molecular mechanism of the regulation of growth by estrogens and glucocorticoids in human and rat breast cancer cell lines.

APPROACH: The MCF 7, ZR 75, RBA and NMU cell lines (kindly provided by Dr. M. Rich, M. Lippman and P.C. Chan respectively) are responsive to estrogens, antiestrogens and glucocorticoids. We would like to determine whether these hormones are stimulating cell proliferation directly or via growth factors secreted by these cells. In a first step, the protein and peptides induced specifically by the hormones will be characterized. In a second step, the effect of these proteins on cell

growth will be tested in cells growing in a chemically defined medium. These studies might help to improve the control of the growth of breast cancer.

56. PROLACTIN RECEPTOR REGULATION IN CULTURED MAMMARY CELLS

Costlow, M. E., Edwards, H. H., St. Jude Childrens Research Hospital, Dept. of Biochemistry, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee*, 38101, United States of America

The objective of the proposed research is to characterize prolactin-receptor regulation in normal rat mammary tissue and in hormone-dependent rat mammary carcinoma. Using primary cultures of prolactin-stimulated normal mammary gland and DMBA-induced, rat mammary tumors which retain prolactin receptors and prolactin responsiveness, we will investigate (1) the direct effects of insulin, progesterone, androgen and estrogen on the level of prolactin-receptor sites, (2) the autoregulation of prolactin receptors, (3) the cellular location of prolactin and prolactin receptor sites from autoradiographic and immunocytochemical studies, and (4) any cell-cycle-specific changes in prolactin receptors. We will determine if the activities of guanyl cyclase and ornithine decarboxylase and the uptake of alpha aminoisobutyric acid are influenced by prolactin and correlated with the number of prolactin receptors and cell growth. The results of these studies should provide a more complete understanding of the mechanism by which prolactin controls the growth of normal and hormone-dependent neoplastic mammary tissue.

57. REGULATION OF ESTROGEN RECEPTORS IN HUMAN BREAST CANCER

Rosner, A. L., Beth Israel Hospital, Dept. of Pathology, *330 Brookline Ave., Boston, Massachusetts*, 02215, United States of America

This project will demonstrate the conditions under which a variety of ligands (metabolites and antiuterotrophic agents) regulate the estrogen dependence of target tissues. The results will be applied as both a means of predicting the hormone responsiveness of a target tissue and a novel criterion on the basis of which the mechanisms of estrogen responsiveness (and thus of the regulation of hormonally induced growth) of normal and tumor target tissues may be distinguished. This investigation is focused on the estrogen receptor as a likely site for the functioning of such controls, taking into consideration much information that indirectly suggests that the estrogen receptor is an allosteric protein. This protein is capable of both homotropic interactions, binding to estrogens in a cooperative manner, and heterotropic interactions, specifically responding to certain ligands which are not steroidal in structure yet which profoundly affect the binding of estrogens to their receptor. This approach is already revealing significant differences between the normal and tumor target cells with regard to the cytoplasmic estrogen receptor. This permits the evaluation of how the alteration of an early response of target cells to steroid hormones could lead to deranged cellular differentiation in neoplastic transformations.

58. HORMONAL CONTROL OF METASTASIS

McGrath, C. M., Soule, H. D., White, A., Michigan Cancer Foundation, Dept. of Biology, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

Our objective is to determine why some breast cancer cells are responsive to hormones and endocrine therapy and others are not. We are using cloned lines of MCF-7, human breast carcinoma cells, which are dependent upon estrogen for growth as tumors in athymic mice, to determine the attributes of the implantation site and the malignant cell that determine hormone responsiveness and the effects of chemotherapy on that responsiveness. Our experimental approach combines cell culture and tumor histogenesis in athymic mice where quantitative data on cell response to hormones and their antagonists are applied in an attempt to modulate tumor growth in vivo.

C. HORMONE RECEPTOR STUDIES

59. NUCLEAR ESTROGEN RECEPTORS IN BREAST CANCER

Horwitz, K. B., University of Colorado, Medical Center, School of Medicine, Dept. of Medicine, *4200 E. 9th Ave., Denver, Colorado*, 80262, United States of America

Estrogen receptors (ER) are closely involved in the processes which regulate growth and regression of breast cancer cells. It is believed that estrogens and their antagonists bind receptors and that the resultant complexes bind to specific nuclear acceptor sites and effect appropriate genes. It is the objective of this proposal to study in detail the nuclear loci of estrogen receptor action contrasting the effects of estradiol and antiestrogens on intranuclear receptor localization and on nuclear receptor turnover or processing with a view to demonstrating the site of divergence between estrogen and anti-estrogen action. These studies will proceed along three lines: (1) In acutely (5 min. to 5 hrs.) estradiol treated human breast cancer cells in tissue culture (MCF-7) we will trace the fate of the nuclear receptor by studying the roles of proteolysis, recompartmentalization and loss of hormone binding capacity. This will be done with parallel studies using routine receptor binding assays and with ER immunoassays. Monoclonal anti-ER antibodies will be produced by fusion of mouse myeloma cells to spleen cells from human ER immunized mice. (2) We will use actinomycin D and other inhibitors to probe the nature of ER interactions with chromatin using radioligand binding assays and immunoassays. (3) We will contrast these studies on estradiol with those on antiestrogen bound nuclear receptors to show whether antagonist properties result from aberrant nuclear receptor processing or binding to inappropriate chromatin sites.

60. STEROID BINDING IN BREAST CANCER BY IMMUNOFLUORESCENCE

Gaetjens, E., Pertschuk, L. P., Kim, D. S., Brigati, D. J., Carter, A. C., State University of New York, Downstate Medical Center, School of Medicine, Dept. of Pathology, *450 Clarkson Ave., Brooklyn, New York*, 11203, United States of America

A successful immunofluorescence technique for detection of estrogen receptor in human breast cancer has been developed. The method is based on the use of an estradiol polymer bound to the specific receptor on frozen sections of tumor. The polymer acts as a large antigen target for subsequent binding of antibody and labeled conjugate. We propose to synthesize water soluble polymers of progesterone, testosterone and glucocorticoid hormones suitable for use in immunofluorescence studies designed to detect specific receptor for these steroids in human mammary carcinoma. Results obtained by immunofluorescence will be compared with those of conventional biochemical assay and correlated with clinical response to therapeutic hormonal manipulations.

BIBLIOGRAPHIC REFERENCES: Pertschuk, L.P., Zava, D.T., Gaetjens, E., Macchia, R.J., Brigati, D.J. and Kim, D.S.: Detection of androgen and estrogen receptors in human prostatic carcinoma and hyperplasia by fluorescence microscopy. Res. Comm. Chem. Pathology and Pharmacology, 22, 427, 1978.

61. INVESTIGATION OF ESTROGEN-NITROSOUREA CONJUGATES WITH POTENTIAL SPECIFIC ANTITUMOR ACTIVITY

Lam, H. P., Manitoba Inst. of Cell Biology, *700 Bannatyne Ave., Winnipeg, Manitoba*, R3E 0V9, Canada

OBJECTIVE: This project aims to test the hypothesis that specific antitumor activity may be achieved by taking advantage of the presence of receptors with specific binding properties, which might result in selective accumulation of cytotoxic agents in ER-positive mammary tumors.

APPROACH: Some estrogen-nitrosourea conjugates will be synthesized. They will be tested against ER-positive and ER-negative mammary tumor cell lines in vitro and in vivo.

62. A MICROMETHOD FOR STEROID HORMONE RECEPTOR ASSAY USING LOW BACKGROUND GAS COUNTER

Casagrande Vainio, M., Parvinen, M., Soini, E., Abo Akademi, Dept. of Biochemistry, *20500 Turku 50, Finland*

The determination of steroid hormone receptor concentrations in human breast cancer tissue is often difficult because of the small amount of tissue available for the analysis. Instead of a conventional open biopsy it would, in many cases, be advisable to use nontraumatic and rapid fine needle biopsy. The limiting factor in receptor assay may, in such cases, be the relatively low counting efficency and the high background of the usual liquid scintillation counter. I would therefore like to explore the possi-

bilities of applying high sensitivity tritium counter in receptor assays of microsamples.

Immediately after removal from the patient, the tissue samples are cooled to 0 degrees C, washed and rinsed from fat and other clearly noncancerous components. According to the method described by Garola and McGuire (1977), the cytosol extract containing the hormone receptors is incubated with tritiated oestradiol. An aliquot of the supernatant is evaporated and radioactivity is measured with a gas counter.

A sensitive automatic gas counter for tritium has been constructed with ten parallel counting channels, a background of 0.6 cpm and a constant quench-free counting efficiency of 61 percent. Biological samples, weighing up to 2 mg dry weight, are sealed in small glass ampules and combusted at 650 degrees C for 60 min. in the presence of zinc powder and anhydrous sodium carbonate. Tritium is converted into gas form during this procedure. The gas counter has more than 50 times greater sensitivity than a usual liquid scintillation counter used to measure tritium.

Preliminary results show that the gas counter is a potential tool for steroid hormone receptor assay when small amounts of tissue are available, e.g., after fine needle aspiration biopsy.

63. ROLE OF PROLACTIN RECEPTORS IN HUMAN BREAST CANCER

Rae Venter, B., University of Texas, Medical Branch at Galveston, School of Medicine, Dept. of Surgery, *300 University Blvd., Galveston, Texas,* 77550, United States of America

The research proposed in this project is directed towards understanding the possible role of prolactin and prolactin receptors in human breast cancer. Preliminary studies by the applicant show that seventy-five percent of the tumors have greater than 2.5 fmoles/mg protein of prolactin receptors; twenty percent of these tumors have prolactin receptors but no estrogen receptors. These data suggest that in at least some tumors, estrogen may not be important for tumor growth. Serum estrogen and prolactin will be measured by radioimmunoassay; prolactin receptors will be measured using 125-I-iodo human prolactin on partially purified membranes from breast tumors, non-malignant breast tissue and normal breast tissue; estrogen receptors will be measured on tumor cytosols. The clinical responses to endocrine ablative procedures of patients whose tumors have both estrogen and prolactin receptors as opposed to only one receptor will be compared. The prolactin receptor concentrations of malignant vs. non-malignant tissue from the same patient will also be compared. Prolactin receptors from normal, non-malignant and malignant breast tissues will be characterized in terms of binding kinetics and hydrodynamic properties.

64. RECEPTOR STUDIES IN HUMAN BREAST CANCER

Hollander, V. P., Joint Diseases North General Hospital, Research Institute for Skeletomuscular Diseases, *1919 Madison Ave., New York, New York,* 10035, United States of America

We have demonstrated that estradiol receptor from two lines of rat mammary tumor MTW9 differ by chromatographic, kinetic and DNA binding properties. MTW9-MtT does not regress after ovariectomy, MTW9-P does regress. MTW9-MtT, the rat mammary tumor grown by coimplantation of the mammosomatotropic tumor MtTW10, shows ovariectomy-induced regression when MtT is resected. MTW9-MtT tumor cytosol receptor shows much less binding to DNA than does receptor from MTW9-P; after resection of MtT, DNA binding is similar. These data suggest that the differences between tumor receptors observed in our animal model may allow differentiation between tumors which have estradiol receptor and either regress or fail to regress after ovariectomy.

We propose to study human breast cancers which contain estradiol receptor by the techniques described which differentiate responsive from non-responsive tumors. Differences in receptor behavior would then be correlated with response to endocrine treatment. If such a study of estradiol receptors in mammary cancer would identify hormone-responsive tumors, current therapy would be improved.

65. INVESTIGATION OF ESTROGEN-NITROSOUREA CONJUGATES WITH POTENTIAL SPECIFIC ANTITUMOR ACTIVITY

Lam, H. P., Manitoba Inst. of Cell Biology, *700 Bannatyne Ave., Winnipeg, Manitoba,* R3E 0V9, Canada

OBJECTIVE: This project aims to test the hypothesis that specific antitumor activity may be achieved by taking advantage of the presence of receptors with specific binding properties, which might result in selective accumulation of cytotoxic agents in ER-positive mammary tumors.

APPROACH: Some estrogen-nitrosourea conjugates will be synthesized. They will be tested against ER-positive and ER-negative mammary tumor cell lines in vitro and in vivo.

66. STEROID RECEPTORS AND INDUCIBLE PROTEINS IN BREAST TUMORS

Duffy, M. J., O'Connell, M., St. Vincent's Hospital, Dept. of Nuclear Medicine, *Elm Park, Dublin, Ireland*

OBJECTIVE: The aim of this project is to determine if estradiol inducible proteins are better markers for hormone dependent breast cancer than the estradiol receptor alone.

APPROACH: Estradiol receptors and the estradiol inducible protein (peroxidase and progesterone) receptors are being measured in both normal animal tissues and breast tumors. The correlation between the presence of these parameters in human breast cancers and response to endocrine therapy is being investigated. It is hoped to determine if the presence of the inducible proteins adds to the predictive ability of the estradiol receptor.

67. BIOCHEMISTRY AND IMMUNOENDOCRINOLOGY OF THE OESTROGEN RECEPTOR FROM DMBA-INDUCED MAMMARY TUMORS OF THE RAT

Griffiths, K., Davies, P., Read, G. F., University of Wales, Welsh National School of Medicine, Tenovus Inst. for Cancer Research, *34 Newport Rd., Cardiff CF1 1XP, Wales, United Kingdom*

OBJECTIVE: It is proposed to use purified oestradiol-receptor protein: (a) to develop antisera to the receptor protein for improved estimation of receptor in tissue extracts and for the establishment of immunohistochemical techniques for localization of receptor; and (b) to study its mechanism of action in cell-free systems, reconstructed in vitro from purified components of the genetic regulatory apparatus.

APPROACH: Further development of this work could provide an assay of great potential for clinicians concerned with the management of patients with breast cancer. It is now necessary to produce greater quantities of purified receptor protein, so that this material and the antiserum it produces may be fully characterized. Two interrelated approaches are to be used: (1) perseverance with DMBA-induced tumors to obtain significant amounts of monospecific immunoglobulin, and (2) use of the immunoglobulin to develop an affinity matrix to purify receptor in greater yield from a larger, more productive source - large animal uteri.

It is also envisaged that a purified receptor protein and an antiserum developed against it would be useful in several respects, apart from improving receptor measurement. The purified protein would be invaluable in studies on the mechanism of steroid hormone action, particularly in investigations of its interaction with genomic constituents involved in the repression and depression of transcription. An antiserum to the receptor protein would be incorporated into a scheme of work devised to follow the movement of the receptor protein through the target cell; this and the precise localization of the receptor could be achieved by immunocytochemical techniques.

68. ENDOCRINE RESPONSIVENESS IN PATIENTS WITH BREAST CANCER

De Kastner, M. R., Schettino, A. M., Instituto Nacional do Cancer, *Caixa Postal 1675-ZC-00, 20000 Rio de Janeiro, R.J., Brazil*

There is considerable evidence that the actions of all steroid hormones on their target cells are mediated, at least in part, through their interaction with receptor proteins. Since conventional methods at the present time for biochemical receptor assay are expensive and require preparation of tumor cytosol by ultracentrifugation, radiolabeled chemicals and scintillation counting, it is vital that methods should be developed for the detection of these receptors suitable for performance in the average community hospital

The purpose of this preliminary study is: 1) To explore the possibility of an immunofluorescent staining method that can be applied to surgical specimens from breast cancers. 2) To study the heterogeneous tumor cell populations in terms of ER and

PgR levels. 3) To demonstrate the real significance and role of ER and PgR measurement. 4) To evaluate the correlation between the presence of tumor ER and PgR and the response to endocrine therapy.

69. A STUDY OF HORMONE RECEPTORS IN TUMOR TISSUE

Skinner, L. G., Barnes, D. M., Lloyd, E. J., Ribeiro, G. G., Bush, H., Paterson Lab., Dept. of Clinical Research, *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVES: (1) To increase the value of receptor assay in predicting hormone responsiveness of breast tumor by improvement and simplification of our present methods for estimating cytoplasmic oestradiol and progesterone receptors and nuclear oestrogen receptors, and to extend the scope of receptor analysis by inclusion of additional hormone receptors. (2) To continue and extend clinical pilot studies on breast carcinoma to provide information on: (a) receptor status and correlation with hormone therapy, (b) relationship between receptor status and response to cytotoxic chemotherapy, (c) usefulness of receptor profile of primary tumor as a predictor of hormone responsiveness of subsequent metastases, (d) effects of chemotherapy, radiation, hormone and anti-oestrogen treatment on receptor site status, and (e) relationship between receptor status and stage of disease.

APPROACH: Current methods of assaying receptors in general are hampered by inavailability of sufficient tissue on many occasions. An attempt will be made to develop more sensitive methods for the assay of receptor sites based on the availability of specific antibody to the receptor protein: These would include use of fluorescently labelled antibody in tissue slices and radioimmunoassay in cytosol. The first step involves isolation and purification of oestradiol receptor from human tumor tissue, using as starting material RE-c positive primary breast tumor obtained at mastectomy. After characterization, the receptor complex will be used to elicit antibody. Alternative methods which may prove to be more sensitive e.g., isoelectrofocussing in various matrices, are also being examined.

PROGRESS: The addition of nuclear receptor (RE-N) assay appears to reinforce the greater precision of prediction of response to hormone therapy in advanced breast cancer; this method involves measuring progesterone receptor as well as oestradiol receptor in the tumor cytosol.

Patients with RE-c positive RP-c positive primary tumors have been shown to have a longer disease-free interval than patients with RP-c negative tumors, irrespective of whether these were RE-c positive or RE-c negative. Both cytoplasmic assays and more recently the additional RE-N assay have been carried out on the tumors from 400-500 patients at mastectomy who are being followed for recurrence and subsequent response.

70. STEROID HORMONE RECEPTOR CONTENT AND PREDICTIVE TEST FOR CHEMOTHERAPY OF BREAST CANCER

Kaufmann, M., Klinga, K., Runnebaum, B., Kubli, F., University Hospital, Dept. of Gynecology, *Voss Str. 9, 6900 Heidelberg 1, Federal Republic of Germany*

OBJECTIVES: a) Information on biochemically prognostic factors relevant to selection of patients for adjuvant postoperative therapy regimens. b) Planning of individual hormonal and/ or cytotoxic chemotherapy in breast cancer.

APPROACH: Hormone receptor assay (17-beta estradiol (ER), progesterone (R 5020) (PgR)-receptor): Receptor determination is done by the dextran charcoal adsorption technique (according to EORCT, 1973). Definition of receptor content (fmol/mg cytosol protein): ER (PgR)-negative: less than 5 (less than 20), -poor: 5-10 (20-50), -rich: greater than 10 (greater than 50).

In vitro chemoresistance test: Native, mechanically isolated tumor cell suspensions were tested for a common primary chemoresistance, using a simple standardized in vitro test system without trypsinizing procedures. Therefore the effect of adriamycin (ADM; 10 to the 4th power - 10 to the minus first power mg/ml) as a non-cycle specific compound will be studied on the 3H-uridine (UdR) incorporation in trichloracid precipitable cell material. Definition of chemoresistance: reduction of UdR-incorporation by ADM (10 to the minus 2nd power mg/ml) less than 25% compared with control values.

PROGRESS: Tissue of primary untreated breast cancer of different histology and tumor stage without distinct metastases was investigated in 53 patients (13 pre-, 40 postmenopausal): ER (PgR)-negative - 36% (54%), ER (PgR)-poor - 11% (20%), and ER (PgR)-rich - 53% (26%). There were more receptor-rich cases in postmenopausal, as compared to premenopausal, women. Out of a total number of 19 ER-negative cases 8 (42%) were chemoresistant (PgR: 10/19 equals 53%) in comparison to 24/28 equals 86% of the ER-rich cases (PgR: 8/9).

Lack of ER- and/or PgR-receptors in primary breast cancer tissue is associated in vitro with increased response to cytotoxic drugs.

71. CLINICAL USEFULNESS OF HORMONE RECEPTOR ASSAY IN ENDOCRINE THERAPY AND CHEMOTHERAPY OF ADVANCED BREAST CANCER

Nomura, Y., Kondo, H., Yamagata, J., Kanda, K., National Kyushu Cancer Center, Dept. of Breast Surgery, *Ohaza-Notame 595, Minami-ku, Fukuoka City 815, Fukuoka, Japan*

OBJECTIVE: To determine the value of hormone receptors in breast cancer as a tool for predicting response to the endocrine therapy and chemotherapy.

APPROACH: The presence or absence of estrogen (ER), progesterone (PgR) and androgen (AR) receptors in tumors were correlated with response to the major endocrine ablation therapy, and with response to chemotherapy.

In order to obtain higher response rate in the endocrine therapy, some clinical and laboratory variables of the patients with advanced breast cancer were estimated concomitantly with the results of ER assay, using the multivariate analysis.

PROGRESS: Er, PgR, and AR were shown to be positive in 54.5% (204/375), 26.6% (54/203), and 24.8% (35/145), respectively. The patients with ER-negative tumors did not respond to the major endocrine ablation therapy (response rate: 0/26). On the other hand, about 60% of the patients with ER-positive tumors responded well to the therapy (23/38). Much higher response rate could be obtained by a multivariate analysis from clinical data of liver, bone metastases, and urinary 17-KS/17-OHCS ratio in ER-positive cases.

Correlations between the combination of presence of hormone receptors and response to endocrine and chemotherapy are studied.

72. HORMONE RECEPTORS AND ACTION IN HUMAN BREAST CANCER

Fazekas, A. G., MacFarlane, J. K., Montreal General Hospital, Surgical Laboratories, *1650 Cedar Ave., Montreal, Quebec,* H3G 1A4, Canada

OBJECTIVE: To predict the response to endocrine ablation or additive hormone therapy with greater accuracy in advanced breast cancer.

APPROACH: The hormone receptor profile for a number of steroid hormones is determined in primary and metastatic breast cancer. Methods for the characterization and quantitative measurement of estradiol, cortisol, dexamethasone and progesterone receptors are routinely used. In addition the nuclear binding of estradiol is studied. The presence or absence of these receptors is correlated to responses to endocrine treatments.

PROGRESS: Estradiol receptors were detected in 50% of 650 cases in significant amounts, in the cytosol of the tumors. Nuclear binding was found in 75% of these cases; the remainder were without detectable translocation of estradiol. Glucocorticoid receptors were found in 23% of all cases, while a transcortin-like cortisol binding protein was detected in over 95% of all cases studied. Approximately one-third of cases showed progesterone binding activity. Studies indicate that some tumors have receptors for a number of steroid hormones indicative of multiple hormone dependency. A significant proportion of ER positive tumors show no nuclear binding.

73. CYTOCHEMICAL STUDIES ON HORMONAL RECEPTORS IN BREAST CANCER

Tremblay, G., Shyamala, G., Buell, R. H., McGill University, Inst. of Pathology, Dept. of Pathology, *3775 University St., Montreal, Quebec,* H3A 2B4, Canada

OBJECTIVE: Biochemical assays of mammary carcinoma tissue for estrogen receptor (ER) provide quantitative data allowing for the prediction of patient response to hormonal therapy but provide little information on the distribution of ER and

fail to predict the observed clinical response in about one-third of patients. Recently, cytochemical techniques were developed demonstrating a heterogeneous population of ER positive and negative tumor cells within many mammary carcinomas. This cellular diversity, not evident in biochemical assays, suggests a partial explanation for unexpected therapeutic failures. The object of this project is to develop techniques which will permit the differentiation on tissue sections of ER positive and negative cells in human mammary carcinomas. Special emphasis will be placed on the distribution of ER in atypical (pre-cancerous) lesions and the various histologic patterns of primary breast carcinoma. The long range plan of the project is a correlation of the cytochemical studies, biochemical assays and the biologic evolution of the carcinoma.

APPROACH: To identify ER positive and negative cells in tissue sections, dry-mount or thaw-mount autoradiography and a peroxidase anti-peroxidase immunocytochemical technique will be used. The accuracy of the methods will first be verified by studying the distribution of ER in the uterus of BALB/c mice after in vitro incubation with or without specific inhibitors and comparing this with known results obtained from in vivo injection of 3H-estradiol. Once characterized, the methods will be applied to a study of human mammary carcinoma.

PROGRESS: The techniques of dry-mount and thaw-mount autoradiography have been successfully used to demonstrate ER positive cells in the uteri of BALB/c mice after in vivo injection of 3H-estradiol. In vitro incubation schemes are being tested which yield results comparing favorably with those observed after in vivo injections. Preliminary results from the incubation of several human breast cancers have also been obtained. For the immunocytochemical technique, the antigen 17-beta-estradiol-6-(O-carboxymethyl) oxime-bovine serum albumin has been synthesized and used for immunization of rabbits. The antisera obtained are being tested for titre and cross-reactivities. In addition, experiments are being done to localize ER in tissue previously studied by autoradiography.

74. MAMMARY AND LIVER PROLACTIN RECEPTORS

Ebner, K. E., Church, W., University of Kansas, Medical Center, School of Medicine, Dept. of Biochemistry, *39th St. & Rainbow Blvd., Kansas City, Kansas*, 66103, United States of America

Prolactin receptors are being purified from rabbit mammary glands and rat liver by solubilization by detergents and subsequent purification with various types of affinity chromatography using concanavalin A, PCMB, amino acids and reduced and alkylated prolactin. Zwitterionic detergents are most useful for purification since they reduce non-specific binding and interfere less with protein assays.

75. MAMMARY CANCER AND HORMONE INDUCED RESPONSES

Clark, J. H., Hardin, J. W., Baylor College of Medicine, School of Medicine, Dept. of Cell Biology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The major goal of this proposal is to establish the qualitative and quantitative relationships between estrogen receptors (type I sites) and secondary estrogen binding sites (type II sites) in mammary tumor tissue. Methods for measurement and evaluation of binding sites in cytosol and nuclear compartments will be examined in hormone dependent and independent mammary tumor lines. The nuclear localization and physical-chemical characteristics of type II sites will be determined. The influence of progesterone on type I and II estrogen binding sites will also be examined and the physiological interactions between these two steroids will be investigated.

76. CHARACTERIZATION OF MAMMARY GLUCOCORTICOID RECEPTOR

Markland, F. S., Rho, J. H., Dunkerton, L. V., Howard, E. B., Li, M. P., Pirkle, H., University of Southern California, School of Medicine, Dept. of Biochemistry, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

The objective of this study is to isolate and characterize the glucocorticoid receptor from a hormonally sensitive target organ, the lactating mammary gland. Our preliminary studies have shown lactating goat mammary tissue to be a good source of this protein. We have developed purification procedures that enable us to prepare a highly purified glucocorticoid receptor. The purified glucocorticoid receptor is being characterized physiochemically and the topography of the steroid binding site is being studied by the use of radioactive affinity-labeling steroid derivatives and site-specific protein modifying agents. Binding of the purified 'activated' steroid-receptor complex to DNA-cellulose or nuclei isolated from target organs will be utilized to identify physiologically important cytoplasmic steroid binding macromolecules. The effect on nuclear binding of modification of specific amino acid residues in the receptor will be studied in order to identify nuclear attachment sites of the receptor protein. Additionally, the purified receptor will be used for the production of an antiserum that may be helpful in the development of sensitive immunoassays to detect steroid hormone receptors in biopsy specimens from women with breast cancer.

77. ENDOCRINE TUMORS - STEROID RECEPTORS AND THERAPY

Rosen, F., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

The overall goals of this proposal are to study the levels and regulation of steroid receptors in endocrine-responsive tumors and thus to improve the therapy of these tumors with steroids, to develop and advance the use of antisteroids, and to evaluate treatments that might prevent muscle wasting that occurs in the tumor-bearing host. The specific objectives are: (1) to relate the response to therapy with steroids to the endogenous and regulated levels of steroid receptors in estrogen-dependent and -independent mammary tumors; (2) to initiate steroid receptor studies and therapeutic trials with the transplantable androgen-dependent rat tumor R-3327; (3) to stimulate the development of antisteroids for use in the treatment of endocrine-responsive tumors; (4) to correlate chemical structure and biological activity of steroid analogues, with particular emphasis on analogues of progesterone and other progestins; (5) to study the basis for resistance of lymphoid tumors to glucocorticoids and other endocrine tumors to steroid therapy; and (6) to explore the possibility that androgen or progesterone analogues, by competing with glucocorticoids for receptors in muscle, can alleviate muscle wasting in advanced cancer.

BIBLIOGRAPHIC REFERENCES: Ip, M.M., Milholland, R.J., Kim, U. and Rosen, F. Mammary Cancer: Selective Action of the Estrogen Receptor Complex. Science, 203, 361-363, 1979. Kaiser, N., Mayer, M., Milholland, R.J. and Rosen, F. Studies on the Antiglucocorticoid Action of Progesterone in Rat Thymocytes. J. Steroid Biochemistry, 10: 379-386, 1979.

78. MAMMARY CANCER AND THE NUCLEAR ESTRADIOL RECEPTOR

Frankel, F. R., Senior, M. B., Sun, L., University of Pennsylvania, School of Medicine, Dept. of Microbiology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

Steroid receptor proteins appear to be regulatory molecules that interact with the cell's genetic material to control gene expression. Some human mammary tumors contain significant amounts of the estradiol receptor, and these tumors often respond to various endocrine therapies with remission of tumor growth. We wish to know more about the nature of the nuclear form of the receptor protein and more particularly, what kind of interaction it shows with chromatin and how hormone analogues may affect this interaction. We have shown that when estradiol is administered to isolated rat uteri or MCF-7 cells, a line of human mammary tumors cells, it binds to chromatin. This estradiol is preferentially released from the chromatin by ingestion with various nucleases with the concomitant production of hormone-bound nucleosomes and hormone-receptor complex bound to a fragment of DNA. Transcriptionally active fractions of chromatin are enriched in estradiol. We now wish to determine (1) whether nucelosomes containing estradiol otherwise differ from the bulk nucleosomes, i.e., whether we can detect the presence of special 'acceptor' proteins or unique DNA sequences; (2) whether the components of active chromatin are different from those of the bulk chromatin; (3) whether steroid receptors occur in patches or singly along the chromatin; (4) whether antiestrogens show similar binding to chromatin, or alter the binding of estradiol; and (5) whether different clones, or mutants of MCF-7 cells show different responses to steroid treatment and, if so, whether the interaction of their

receptors with chromatin will differ from that occurring in wild type cells.

BIBLIOGRAPHIC REFERENCES: Enrichment of estradiol-receptor complexes in a transcriptionally active fraction of chromatin from MCF-7 cells. Richard W. Scott and Fred R. Frankel, Proc. Natl. Acad. Sci. USA 77: 0000 (1980), in press.

79. HORMONE RECEPTOR REGULATION IN MAMMARY TISSUE

Costlow, M. E., Koseki, Y., St. Jude Childrens Research Hospital, Dept. of Biochemistry, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee,* 38101, United States of America

Hormone dependence in experimental breast cancer is a complex phenomenon involving the concerted action of a number of hormones. In many instances, modifications occur in the chain of biochemical events controlling tumor growth and result in a loss or alteration of hormonal control. Although a loss of certain receptors accounts for a loss of hormone responsiveness in some tumors, it is becoming evident that alterations in endocrine regulation of hormone receptors may be a critical determinant of hormone dependence.

We propose to study hormonal regulation of receptors for estrogen, progesterone and prolactin in normal mouse mammary tissue in DDD mice and in a transplantable pregnancy-dependent mouse mammary tumor (TPDMT-4) which is clearly dependent on all these hormones for growth. Receptor assays will be developed to determine the total number of estrogen and progesterone receptors (free plus occupied) in both nuclear and cytoplasmic compartments and the total number of cell-surface receptors for prolactin. The dose-related effects of insulin, estrogen, progesterone and prolactin, singly and in combination, will be examined to determine if there are fundamental - possibly oncogenically relevant - differences in the level of receptors and/or receptor regulation in normal and neoplastic mammary tissue.

These studies should define the relative usefulness of prolactin, estrogen and progesterone receptors as predictive markers of hormone dependence and provide insight into the complex hormonal interrelationships governing hormone-dependent tumors. Definition of key steps in the hormonal stimulatory pathway may ultimately lead to more rational approaches to endocrine therapy for individual breast cancer patients.

BIBLIOGRAPHIC REFERENCES: Koseki, Y., and Costlow, M.E.: A sensitive assay method for total progesterone receptors. Fed. Proc., 38: 483 (1979).

80. STEROID SULFATION AND ESTROGEN BINDING IN BREAST CANCER

Dao, T. L., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

The specific aims are: To correlate the steroid sulfotransferase activity and estradiol binding proteins in the primary tumor at the time of mastectomy with pathological staging of the disease and the rate of recurrence; and to evaluate whether measurements of these biochemical parameters are useful for predicting response to endocrine ablation when metastasis occurs in these patients.

81. ESTROGEN BINDING STUDIES IN OVARIAN DEPENDENT AND INDEPENDENT RAT MAMMARY CARCINOMA

Bodwin, J. S., Cho Chung, Y. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

This study continues to investigate how the specific biochemical phenomenon of estrogen receptor binding relates to the cAMP binding proteins and growth and regression of ovarian dependent and independent tumors.

BIBLIOGRAPHIC REFERENCES: Cho-Chung, Y.S., Bodwin, J.S., Clair, T.: Cyclic AMP Binding Proteins: Role in Ovariectomy Induced Regression of a Hormone Dependent Mammary Tumor. J. Nat'l. Cancer Inst. 60: 1175-1178, 1978. Bodwin, J. S., and Cho-Chung, Y.S.: Dibutyryl Cyclic AMP Mimics The Effect of Ovariectomy on a Dehydrogenase in Hormone Dependent Mammary Tumors. Eur. J. Cancer 15: 443-449, 1979.

82. STEROID HORMONE RECEPTORS IN HUMAN HORMONE-SENSITIVE TUMORS

Galli, M. C., DeGiovanni, C., Grilli, S., Universita degli Studi di Bologna, Istituto di Cancerologia, *via S. Giacomo 14, 40126 Bologna, Italy*

OBJECTIVE: To test a correlation between the presence of estrogen, androgen, progestin and/or glucocorticoid receptors in human hormone-sensitive tumors and the response rate to endocrine treatment.

APPROACH: Cytoplasmic steroid receptors are determined using a slightly modified dextan-coated charcoal (DCC) method (Cancer Lett. 2, 247-158, 1977; IRCS Med. Sci., 6, 418, 1978) in breast, prostate, endometrium, kidney and ovary cancer and related normal tissues. Hormonal plasma levels and histochemical parameters such as: grading, elastosis, casein production, etc., are determined and clinical data are collected.

PROGRESS: Up to now steroid hormone receptors have been determined in about 200 breast specimens, 50 prostate specimens, 100 endometrial biopsies and 20 cases of kidney tumor and 20 cases of ovary neoplasm. Interestingly, estrogen, androgen, progestin and glucocorticoid receptors are present also in normal and neoplastic ovary.

83. OESTROGEN RECEPTORS, PERIPHERAL OESTROGEN LEVELS AND OESTROGEN METABOLISM IN THE TUMOR IN HUMAN MAMMARY CARCINOMA

IngelmanSundberg, A., Carlstrom, K., Theve, N. O., Blomstedt, B., Skoldefors, H., Wilking, N., Sabbatsberg Sjukhuset, Dept. of Obstetrics & Gynaecology, *Dalagatan 9-11, Stockholm, Sweden*

The frequency of mammary tumors containing high levels of oestrogen receptor is far higher in postmenopausal than in premenopausal women, and we have recently shown a clear correlation between high peripheral oestrogen levels and low receptor values. At low oestrogen levels high as well as low receptor values are recorded. In the proposed project we plan to investigate possible correlation between oestrogen receptor values and the changing serum oestrogen levels during the menstrual cycle and before and after oophorectomy. Furthermore we plan to investigate the correlation between oestrogen receptor values and the metabolism of (3H) oestrone sulphate by the tumor tissue in patients with low peripheral oestrogen levels.

84. STUDIES ON ESTRADIOL AND PROGESTERONE RECEPTORS IN MALIGNANT TUMORS WITH SPECIAL REFERENCE TO BREAST CANCER (ABBREV)

Norgren, A., University Hospital of Lund, Dept. of Lung Medicine/Research, *E. Blocket, S22185 Lund, Sweden*

OBJECTIVES: 1) Thorough testing of currently used methods in receptor research with the aim to improve, for instance, yield of receptor, and to make possible the estimation of total concentration of cytosol receptor protein. 2) Elaboration of experimental biological test systems for the study of control mechanisms for receptor synthesis. 3) Study of importance of the different receptor profiles in prospective clinical breast cancer trials with regard to prognosis of the disease and the effect of adjuvant therapy. 4) Estimation of hormone receptor levels in hormone dependent malignant tumors other than breast cancer.

85. STEROID RECEPTORS AND THE ADENYLYL CYCLASE PROTEIN KINASE SYSTEM IN HUMAN MAMMARY TISSUE

Talmadge, K. W., Almendral, A., Wyss, H., University Womens Clinic, Hormone Laboratory, *Basel, Switzerland*

Though a correlation exists between the estrogen-binding capacity of mammary tumors and their growth response to hormone deprivation, recent findings suggest strongly that it is no longer appropriate to classify mammary tumors simply by the presence or absence of estrogen receptors. Boylan and Wittliff reported that some ovarian independent mammary tumors contain also significant amounts of specific estrogen binding activity. Furthermore, it was shown that the action of estrogen in target tissues such as mammary epithelial cells may be affected by the intervention of prolactin, progesterone and alpha-fetoprotein. Therefore, a combination of criteria involving the determination of more than one receptor was proposed to improve the prediction of growth response to endocrine ablation.

However, the role of cAMP as second hormone messenger in the mechanism of estrogen action is not clear. Since cell proliferation and glycogen deposition are the responses of uterine tissue to estrogen, it is very unlikely that cAMP can be a mediator of these hormone-induced effects. Cyclic AMP has been shown to have an antagonistic influence on such cellular events. Recent evidence points out that cAMP and estrogen may counteract each other in the growth control of hormone-dependent tumors such as DMBA-induced mammary tumors.

This research is designed to continue to elucidate the precise role of cAMP as mediator of the adenylyl cyclase protein kinase system for the clinical high risk evaluation of patients with dysplasia and to evaluate the role of cAMP and the adenylyl cyclase protein kinase system as cellular antagonists to estrogen receptors in human mammary tumors.

86. ESTROGEN RECEPTOR (ER) IN NORMAL AND TUMOR TISSUE OF THE BREAST (CORRELATION WITH CLINICAL AND PARACLINICAL PARAMETERS)

Rivadeneyra, J., Gomez, P., Rabago, M., Hospital de Oncologia, Laboratorio de Investigacion Clinica, *Av. Cuauhtemoc 330, Mexico City 7, D.F., Mexico*

OBJECTIVE: ER correlation with spontaneous evolution in patients from whom samples were taken at the time of initial mastectomy.

APPROACH: Cytosol is incubated with increasing quantities of (3H)E2 in duplicate for 18 hours at 4 degrees C. Novit A and grade C dextran are then added for an additional 30 minutes at 4 degrees C, with vigorous shaking. The mixture is then centrifuged, and the supernatant radioactivity is quantitated in a scintillation cocktail. Data are evaluated by Scatchard analysis.

PROGRESS: We have studied 86 malignant, 27 benign and 24 normal samples of tissue. Dextran coated charcoal (DCC) was used to measure ER.

The ER positive values of this group were analyzed. The results were 69%, 52% and 0%, respectively. Mean values for each group were 83.6 plus or minus 12.1 s.e. fm/mg/cp (cytosol protein) and 48.8 plus or minus 14.2 s.e. fm/mg/cp respectively for the malignant and benign groups. The values of the malignant group were divided according to the menstrual status. Results were 64% with a median of 64.9 plus or minus 9.1 s.e. for menstruating patients and 73% with a median of 99.3 plus or minus 16.9 s.e. for the menopausal group, p greater than 0.05.

We also decided to analyze the differences in mean values according to decades; our findings demonstrate that there is a difference between the 21-30 decade and that of 51-60, p less than 0.01, and also between those in the 31-40 age group and those in the 51-60 decade, p less than 0.05. We are now working on the follow-up of this group.

87. RATIONAL DESIGN OF BREAST TUMOR LOCALIZING AGENTS

Katzenellenbogen, J. A., Goswami, R., Heiman, D. F., Kilbourn, M. R., Carlson, K. E., University of Illinois, Urbana-Champaign Campus, School of Chemical Sciences, Dept. of Chemistry, *Urbana, Illinois*, 61801, United States of America

A large number of human breast tumors contain estrogen receptors and, thus, have the ability to concentrate estrogenic substances. In principle, one should be able to utilize this selective uptake mechanism to concentrate a gamma-emitting radiopharmaceutical whose distribution could be monitored by gamma-imaging techniques. Such a procedure would provide a unique, non-invasive diagnostic tool for localizing breast tumors and metastases and determining their receptor content. This project is concerned with the synthesis and evaluation of such receptor-based, selective breast tumor localizing agents.

Halogenated derivatives (fluorine-18, bromine-77 and iodine-123) of various steroidal and non-steroidal estrogens will be prepared. The halogen will be placed at a position consistent with high chemical and metabolic stability and high receptor binding affinity, and the overall structure of the molecule will be designed to maximize its binding selectivity. This will be done by adjusting its structure to optimize its 'merit factor', which is defined as the ratio of its binding affinity for the estrogen receptor to its binding affinity for non-receptor proteins.

We have already prepared homologous series of iodostilbestrols and ring and side chain halogenated hexestrols; some of these compounds have merit factors in the 30-300 range. (The merit factor for estradiol is 100 by definition.) These compounds will be studied in vitro (receptor, serum, control and target tissue binding) and in vivo (tissue distribution and uptake selectivity) in animal model systems (DMBA-induced and R3230AC transplantable mammary tumor-bearing rats) to assess their feasibility as diagnostic agents for human breast cancer.

88. THE MOLECULAR MECHANISMS OF ESTROGEN RECEPTOR TRANSFORMATIONS IN MAMMARY GLANDS

Shyamala, G., Jewish General Hospital, *3755 Cote St. Catherine Rd., Montreal, Quebec*, H3T 1E2, Canada

OBJECTIVE: The objectives of the project are twofold: (1) to investigate in detail the mechanism of activation of cytoplasmic EBP of normal and neoplastic mammary glands and (2) to investigate the biological significance of the activated EBP in the expression of estrogenic effects specific for mammary cells.

APPROACH: The native cytoplasmic EBP, activated cytoplasmic EBP and nuclear EBP are being analyzed for such molecular parameters as sedimentation rates, Stokes radii, frictional ratio, axial ratios and molecular weights. The activated cytoplasmic EBP is to be distinguished from the native EBP by its close similarity to nuclear EBP with respect to its molecular parameters. The hypotheses proposed with respect to the activation of steroid-receptor complexes include roles for temperature, ionic strength and certain cytoplasmic factors. To assess the biological significance of activation of EBP, attempts are being made to correlate the presence of a particular form of EBP with the appearance of a specific estrogen induced end product such as progesterone receptors.

PROGRESS: Studies from our laboratory indicate that: (a) there are two related forms of EBP in the cytoplasm of mammary glands, one of which appears to be identical to the nuclear EBP and thus has been termed the activated EBP; and (b) the activation involves conformational changes resulting in an EBP lower in molecular weight then the native EBP. We have also identified and characterized the progesterone receptors in normal mammary tissues.

89. INTERACTION OF HOST CELLS AND TUMOR CELLS AT THE HORMONE RECEPTOR LEVEL

Brandes, L. J., Manitoba Cancer Treatment & Research Foundation, Dept. of Cell Biology, *700 Bannatyne Ave., c/o U. Manitoba Hlth. Sci. Ctr, Winnipeg, Manitoba*, R3E 0V9, Canada

Estrogen receptor and dexamethasone receptor content of an in vitro breast cancer cell line (MCF7) is determined. Similar determination of dexamethasone receptor content on peripheral blood T cells, B cells and macrophages is also made. Interactions between monolayers of peripheral blood lymphocytes and macrophages are monitored by measuring possible changes in the receptor content per cell (modulation of receptor sites).

Modulation of dexamethasone receptors in peripheral B cells, T cells and macrophages are also measured in this system. Such an approach may give insight into the possible modulation of receptor sites as the result of tumor host contact and may also give insight into the possible mechanism of cell-cell interaction at the hormone receptor level.

90. COMPARATIVE STUDY OF ESTROGEN RECEPTOR ACTIVITY IN MAMMARY CANCER BY RADIORECEPTOR ASSAY AND FLUORESCENCE MICROSCOPY

Remmele, W., Frank, K., Heine, M., Heicke, B., Municipal Hospital, Dept. of Pathology, *Schwalbacher Str. 62, D6200 Wiesbaden, Federal Republic of Germany*

OBJECTIVE AND APPROACH: The purpose of the project is to compare the results of estimation of steroid receptor activity by radioreceptor-assay (Prof. Heicke, Univ. of Mainz-Bioscientia Ingelheim) and by the fluorescence microscopy method of Lee (Amer. J. Clin. Path. 70: 197-203, 1978). The method of Lee is used as originally described as well as in a modified way developed in our laboratory.

PROGESS: Series of experiments has just started; no data are yet available.

91. ESTROGENS AND BREAST CANCER

Chalkley, R. G., University of Iowa, School of Medicine, Dept. of Biochemistry, *Jessup Hall, Iowa City, Iowa,* 52240, United States of America

This research proposal is designed to probe the role of cytoplasmic estrogen receptors in hormone-dependent and -independent rat mammary adenocarcinomas. Specifically, we wish to ask if the receptors play a role in hormone transport into the cell. Further, we wish to assay if the nuclear membrane specific binding site described by us in mature endometrium is also present in hormone-dependent breast tumor cells, especially whether it is absent in cells which contain cytoplasmic receptors but are hormone independent and do not respond by regression upon ovariectomy or hypophysectomy.

In addition, we wish to characterize the membrane site of binding, and we hope to isolate a binding protein and to characterize it chemically. We also hope to determine whether this membrane fraction plays any role in control or modification of either DNA or (more likely) RNA synthesis.

92. STEROID RECEPTORS IN BREAST CANCER AND OTHER TUMORS

Funder, J. W., Prince Henry's Hospital, Medical Research Centre, *St. Kilda Rd., Melbourne, Victoria 3004. Australia*

OBJECTIVE: To determine the incidence of estrogen receptors, progesterone receptors, and androgen receptors, and glucocorticoid receptors in breast cancer, carcinoma of the colon, melanoma and other solid tumors.

APPROACH: To utilize techniques of incubating tumor cytosol with tritiated estradiol, tritiated R-5020, tritiated R-1881 and tritiated dexamethasone, as markers for each of the various classes of steroid receptor.

PROGRESS: In a large series of breast tumors, estrogen and progesterone receptors have been found absent in one third; one third of the tumors have been found to contain estrogen receptors but not progesterone receptors; and one third have been found to contain receptors for both hormones. Studies on androgen and glucocorticoid receptors in breast tumors, and receptors for all four steroids currently being tested in other tumors, have been found at a lower incidence. To date, the small number of tumors so treated precludes statistical evaluation.

93. ESTROGEN RECEPTOR PROTEINS (RATS)

Schneider, S., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

94. PROLACTIN RECEPTOR REGULATION IN CULTURED MAMMARY CELLS

Costlow, M. E., Edwards, H. H., St. Jude Childrens Research Hospital, Dept. of Biochemistry, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee,* 38101, United States of America

The summary for this project is under entry no. 56.

95. REGULATION OF ESTROGEN RECEPTORS IN HUMAN BREAST CANCER

Rosner, A. L., Beth Israel Hospital, Dept. of Pathology, *330 Brookline Ave., Boston, Massachusetts,* 02215, United States of America

The summary for this project is under entry no. 57.

D. OTHER STUDIES RELATED TO THE BIOLOGICAL ROLE OF HORMONES

96. ENDOGENOUS VIRUS AND HORMONES IN MAMMARY CANCER

McGrath, C. M., Jones, R. F., Michigan Cancer Foundation, Dept. of Biology, *110 E. Warren St., Detroit, Michigan,* 48201, United States of America

The objective of work supported by this grant is to determine the role of endogenous (germ line transmitted) MuMTV genes in neoplastic transformation of normal mammary cells under impress of mammotropic hormonal stimulations in vitro.

We have established that a major locus of control over MuMTV gene expression in both normal and malignant BALB/c mammary cells is post-transcriptional. We propose to determine how hormones that promote transformation of mammary cells effect those controls.

We have isolated tumorigenic clones from mammary tumors and non-tumorigenic clones from mammary glands in a specially devised suspension culture system. We propose to transform the non-tumorigenic cells and determine concordant pertubations in MuMTV gene expression. We propose to define the tumorigenic phenotype in terms of altered proliferative responses to mammotropic hormones using these cells in the suspension culture system.

97. PSYCHOENDOCRINE CORRELATES TO BREAST CANCER (HUMAN)

Holland, J. C., Montefiore Hospital & Medical Center, *111 E. 210th St., Bronx, New York,* 10467, United States of America

This is part of a broader project. A summary of this subproject is not available.

98. ORIGIN OF HORMONE DEPENDENT AND INDEPENDENT MAMMARY TUMORS

Bern, H. A., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California,* 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

99. EXOGENOUS HORMONE USE AND BREAST CANCER DEVELOPMENT

Wynder, E. L., American Health Foundation Inc., *320 E. 43rd St., New York, New York,* 10017, United States of America

This is part of a broader project. A summary of this subproject is not available.

100. ESTROGEN BINDING IN BREAST TISSUE (HUMAN)

Schwartz, M. K., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

101. HORMONE DEPENDENCE IN ENDOMETRIAL AND BREAST CANCER

Giannopoulos, G., Davies, J. I., Knapp, R., Harvard University, Boston Campus, Boston Hospital for Women, Dept. of Obstetrics & Gynecology, *221 Longwood Ave., Boston, Massachusetts,* 02115, United States of America

The following investigations are proposed: 1. To quantitate the levels of estrogen and progesterone receptors in normal endometrium, hyperplastic endometrium, endometrial carcinoma, and breast carcinoma. The data will be used to evaluate the correlation of critical levels of estrogen and/or progesterone receptors in endometrial and breast tumors with the patients' response to endocrine therapy. 2. The association of estrogens with endometrial cancer will be examined by measuring estrone and estradiol levels in plasma, whole endometrial tissue and endometrial nuclei, as well as estrone-estradiol interconversions in the endometrium of patients with hyperplasia and carcinoma. 3. The mechanisms involved in the objective remission of endometrial tumors by progestational agents will be investigated by studying the following parameters in tumor tissue before and after progestin therapy: levels of estroger receptors; levels of estrone and estradiol; estrone-estradiol interconversion; uptake and intracellular distribution of estrone and estradiol.

102. HORMONE DEPENDENT AND INDEPENDENT MOUSE MAMMARY TUMOR CELLS

Sluyser, M., Nederlands Kankerinstituut, Div. of Endocrinology, *108 Sarphatistraat, Amsterdam, Netherlands*

OBJECTIVE: To distinguish between hormone dependent and independent mammary tumor cells.

APPROACH: Mammary tumors are induced in ovariectomized GR mice by continuous treatment for 3-4 months with estrone and progesterone. About 85% of the induced tumors are hormone dependent. Serial transplantation in estrone and progesterone treated castrated (020xGR)F1 hybrid mice usually (but not always) leads to a progressive decrease in hormone responsiveness of the transplants. Evidence has been presented that this loss of hormone responsiveness is caused by a decrease in hormone dependent cells in the tumor, relative to the inde-

pendent cells. We can separate the independent cells by grafting tumors in castrated mice which are not treated with hormones, since then only the independent cells grow to form outgrowths. Thus we can compare various characteristics of hormone dependent and independent cells.

PROGRESS: Hormone dependent mouse mammary tumor cells were found to have higher contents of estrogen receptor, progestin receptor and prolactin receptor than independent cells. Differences between these two cell types were also found in their ability to produce the mouse mammary tumor virus (B-particles). Hormone dependent and independent mouse mammary tumor cells were inhibited by cyclophosphamide to equal extents. However, this cytostatic drug inhibited rapidly growing tumors to a higher extent than slowly growing tumors.

We are currently examining other possible markers to distinguish hormone dependent and independent mammary tumor cells.

II. BIOLOGY OF BREAST CANCER

A. MORPHOLOGICAL CHARACTERIZATION OF BREAST CANCER

103. MORPHOLOGY OF THE HUMAN BREAST

Anderson, T. J., Ferguson, D. J., Manton, S., University of Edinburgh, School of Medicine, Dept. of Pathology, *Teviot Pl., Edinburgh EH8 9AG, Scotland, United Kingdom*

OBJECTIVE: To identify morphological changes in breast parenchymal components that can be attributed to the influence of menstrual cycles, parity or physiological aging.

APPROACH: Breast tissue obtained from three sources (surgical biopsy, mastectomy or post mortem examinations) is studied by subgross stereomicroscopy for three-dimensional appreciation of parenchymal morphology. Pattern of development, relationship of lobule to ductal components and degrees of uniformity in normal areas are analysed for relationship to physiological state under categories outlined. Specimens are processed for light microscopy, and electron microscopy from suitable material, for evaluation of (parenchymal) cell composition, type and activity in parenchymal units. Techniques will be developed to allow selection of subgrossly identified parenchymal units of lobule and terminal duct for further study through the full range of microscope magnifications to give more precise biological characterization of these structures. Computer-assisted semi-automated image analysis of tissue sections and X-rays is being performed, the latter for correlative exercises with mammographic patterns.

PROGRESS: Complexity and variability of three-dimensional morphology in parenchyma is now appreciated and methods of comparative analysis are being developed. Cell turnover in lobules, evaluated as division (mitosis) or programmed death (apoptosis), has been demonstrated to show variation related to phases of menstrual cycle. Each is increased around the time of menstruation, but the events appear separated in time and space, with division preceding death.

104. MORPHOLOGY AND BEHAVIOR OF MAMMARY CELLS IN CULTURE (MAMMALS)

Pitelka, D. R., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California,* 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

105. INVESTIGATION OF POSSIBLE CORRELATIONS BETWEEN MORPHOLOGICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF BREAST CANCER

Stenkvist, B., University Hospital, Dept. of Clinical Cytology, *75014 Uppsala, Sweden*

To ascertain possible correlations between the morphologic variables and the established epidemiological risk factors of breast cancer. The results may be applied not only to identify individuals at high risk of breast cancer but may be used prognostically to predict course of disease. This prospective study will consist of two phases: Phase I will consist of a pilot study of histopathologic material from 110 breast cancers from four counties in Sweden utilizing the computerized Central Swedish Cancer Registry. The tumors will be classified histologically according to WHO (Scarrf and Torloni 1968) and Acherman's Classification (1970). Another aim of this project is to make a quantitative evaluation of a number of morphological characteristics by computer analysis of tumor cell populations utilizing a Leitz MPVII scanning photometer connected to a PDP-8 computer with 24K of core, and relate these to the standard classification. Planning for the epidemiologic data will be collected on each patient and computerized. Under Phase II statistical analysis of the data will be made with application of the newly acquired morphologic classification to the epidemiologic variables.

106. MORPHOLOGIC AND EPIDEMIOLOGIC ASPECTS OF BREAST CANCER

Stenkvist, B., Uppsala Universitet, *Box 256, S75105 Uppsala, Sweden*

The Contractor intends to select randomly 100 out of about 1000 imprint specimens from different tumors. The specimens will be interactively analyzed in their cell analysis system, and the result from this processing will be saved and later subjected to careful statistical analysis on a large-scale computer, in order to define a measure characterizing nuclear grade for each one of the selected tumors. He also intends to see if there are any natural categorizations of the tumors from the nuclear-grade point of view. The results from these experiments will be compared with the conventional classification used by pathologists and cytologists. In order to evaluate the importance of the obtained measure of nuclear grading, he intends to relate it to the three- and five-year survival of the patients in a follow-up study.

107. NEOPLASTIC MAMMARY GLAND - STRUCTURE/ FUNCTION

Strum, J. M., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Anatomy, *655 W. Baltimore St., Baltimore, Maryland,* 21201, United States of America

The purpose of the proposed research is to provide information about the structure and specific functions of the various cell types within normal and malignant mammary glands. (1) Mammary tissues from experimental animals (including preneoplastic lesions, hormone-dependent tumors and hormone-independent tumors) will be studied in vivo and in vitro. The fine structure and function of the different cell populations within these tissues will be elucidated, and some cellular responses to specific hormones will be characterized. (2) Human breast tissue will be maintained in organ culture and examined after exposure to specific hormones. Electron microscopy and electron microscopic autoradiography will be employed to characterize the cell types and to examine certain functional alterations that occur in the tissue (e.g., the ability of cells to divide, incorporate amino acids and bind hormones). (3) The ability of preneoplastic and neoplastic mammary tissues to elicit the ingrowth of new capillaries (a process known as mammary tumor angiogenesis) will be evaluated, and the relationship of this process to malignant transformation will be studied in hormone-dependent and hormone-independent mammary tumors. The role of hormonal regulation in tumor angiogenesis has not been studied heretofore; positive results could lead to new therapeutic methods for controlling the growth of human malignancies.

108. BIOLOGICAL CHARACTERIZATION STUDIES OF MAMMARY TUMORS

Bogden, A. E., Mason Research Inst. Inc., *21 Harvard St., Worcester, Massachusetts,* 01608, United States of America

OBJECTIVES: Acquisition of additional human and selected animal mammary tumor and normal cell lines. Cell line characterizations. Screening of human cell lines for M-PMV (or related) antigen. Cryopreservation of and characterization of in vivo established, transplantable human mammary tumors. Characterization of endocrine responsive tumors received from Dr. R. L. Noble, Cancer Research Centre, University of British Columbia. Establishing an indexed comprehensive bibliography of publications resulting from studies utilizing tissues obtained from the animal tumor and human cell culture bank.

109. IDENTIFICATION OF MAMMARY TISSUE

Soloff, M. S., Medical College of Ohio at Toledo, School of Medicine, Dept. of Biochemistry, *Box 6190, Toledo, Ohio*, 43614, United States of America

OBJECTIVES: Purify myoepithelial cells from normal lactating rat mammary gland. Obtain antisera specifically directed against myoepithelial cell antigens. Develop, along with the antisera, as many independent markers as possible to identify, characterize, and quantitate myoepithelial cells in various stages of differentiation in vivo and in vitro, and in mammary neoplasia.

110. CORRELATIONS BETWEEN MORPHOLOGY AND EPIDEMIOLOGY OF BREAST CANCER

Stenkvist, B. G., Westman Naeser, S., Bengtsson, E. N., Eriksson, O. L., Jarkrans, T., Nordin, B. A., University Hospital, Dept. of Clinical Cytology, *75014 Uppsala, Sweden*

Within a clearly defined area and population (four Swedish counties) 181 breast cancer cases occurred during five months. Epidemiological and morphological data were obtained from 179 of these patients and from 179 exactly age-matched population controls. The tumors were characterized by computerized cytometry of the tumor cell population and by conventional morphology, resulting in an objective atypia measure. Comparison between patients and controls showed no significant differences in age at menarche, age at first birth, age at menopause, number of children, height, obesity, or frequency of diabetes or hypertension. These results differ from most earlier reports, possibly because the controls here were selected from the whole female population instead of hospitalized patients. Breast cancer in the family and prior breast disease in the study persons were significantly higher in the patient group. Data on thyroid hormone levels suggested that altered thyroid function in the patients was probably due to a change in the peripheral conversion of thyroxin. The patients had higher serum concentrations of estrone, androstenedione and testosterone, and a lower level of sex-hormone-binding globulin. No obvious differences in the epidemiological characteristics could be discerned among different conventional morphological subgroups. The computerized cytometry value was positively correlated with biological parameters such as mitotic rate and had significantly better reproducibility than subjective systems for classification or grading. The patients have been followed up for the first thousand days post-mastectomy. A 'malignancy grade' for breast carcinomas has been developed based on image analysis and pattern recognitions. Patients with tumors over the mean value had 39% recurrences within the first thousand days of observation, patients with tumors below this mean value - 0%. This 'high-risk' index for the first thousand days of follow-up was superior to others.

111. MORPHOGENESIS OF THE MAMMARY GLAND WITH SPECIAL REFERENCE TO INVASIVENESS

Wolpert, L., Tickle, C. A., Harrison, C. J., University of London, Middlesex Hospital Medical School, Dept. of Biology as Applied to Medicine, *Cleveland St., London W1P 6DB, England, United Kingdom*

OBJECTIVE: To determine the cellular mechanisms involved in the epithelial invasion of the embryonic mammary gland mesenchyme during normal morphogenesis to provide insights into the invasiveness of mammary carcinomas.

APPROACH: It is proposed to determine the ultrastructural characteristics at the tips of invading epithelial tongues of developing mouse mammary glands at different stages of development. Particular attention will be given to the presence or absence of the basement membrane. The pattern of cell division in the epithelium will be examined both in vivo and in organ culture. Fibrinolytic activity of the epithelium will be assayed. The invasiveness of the epithelium will be assayed in the early chick limb bud.

B. CELL MEMBRANE AND SURFACE PROPERTIES ASSOCIATED WITH BREAST CANCER

112. THE ROLE OF BASEMENT MEMBRANE COLLAGEN IN NORMAL AND NEOPLASTIC MAMMARY EPITHELIAL GROWTH

Wicha, M., Kidwell, W., Kiotta, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

In vivo mammary ductal and alveolar cells rest on a basement membrane containing type IV collagen. The objective of this project was to study the role of basement membrane collagen in normal and neoplastic mammary growth. An in vitro system for the primary culture of normal mammary epithelium and DMBA induced mammary tumor cells was developed. We have shown that normal mammary epithelium in culture synthesizes basement membrane collagen and that process is under hormonal control. We will now demonstrate that normal mammary epithelium preferentially attaches to basement membrane collagen compared to stromal collagen. Attachment, spreading and growth of these cells on stromal collagen requires the synthesis of basement membrane collagen and can be blocked by the proline analog cis-hydroxyproline. Unlike normal epithelium mammary fibroblasts or tumor cells show no preferential attachment to any collagen type. Studies were begun to correlate these in vitro findings with the collagen requirements for growth of normal mammary epithelium and DMBA induced mammary tumors in vivo.

113. BASEMENT MEMBRANE COLLAGEN SYNTHESIS AND DEGRADATION IN THE NORMAL AND NEOPLASTIC MAMMARY GLAND

Liotta, L. A., Wicha, M., Garbisa, S., Kidwell, W., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We have purified and characterized mammary basement membrane collagen type IV, and studied its synthesis and turnover in normal and neoplastic mammary gland.

BIBLIOGRAPHIC REFERENCES: Liotta, L.A., Wicha, M., Foibart, J.M., Rennard, S. and Kedwell, W.R.: Basement membrane collagen synthesized by mammary epithelium. J. Cell Biol. 79: 146, 1978. Wicha, M.S., Liotta, L.A., and Kidwell, W.R.: Effects of free fatty acids on the growth of normal and neoplastic rat mammary gland epithelium. Cancer Res. 39: 425-435, 1979.

114. PLASMATIC MEMBRANE AND ITS ROLE IN THE RADIORESISTANCE AND RADIOSENSITIVITY OF BREAST CANCER

Castro de la Mata, D., de McCarthy, O. C., Zaharia Bassan, M., Caceres Graziani, E., Instituto Nacional de Enfermedades Neoplasicas, *Avenida Alfonso Ugarte #825, Lima 1, Peru*

OBJECTIVE: To demonstrate that plasmatic membrane has a definite role in the radioresistance and radiosensitivity of breast cancer. The results will be used to decide which patients will and which will not respond to radiotherapy.

METHOD: We believe that the only way to determine the validity of the previous statement is by electron microscope study of specimens, before and after radiotherapy treatment.

PROGRESS: At the moment, we are studying four patients: a) Breast cancer 'with membrane,' b) breast cancer 'with a few cells with membrane,' c) breast cancer 'with a lot of cells with membrane,' and d) breast cancer 'without membrane.'

115. MOLECULAR MECHANISMS OF THE FORMATION AND DISRUPTION OF THE EPITHELIAL BASAL LAMINA

Bernfield, M. R., David, G., Stanford University, School of Medicine, Dept. of Pediatrics, *Stanford, California*, 94305, United States of America

The basal lamina is critical for the generation and maintenance of epithelial morphology and basal lamina disruption is

thought to be a key step in carcinomatous invasion and metastasis.

As a model we will use low passage cultures of normal mammary epithelial cells, which form a basal lamina when cultured on fibrillar collagen, and high passage cultures of cells of the same origin, which display some in vitro characteristics of transformation and lack the ability to form a basal lamina. These cell types will be compared with regard to the physicochemical nature of the proteoglycans made, the possible associations of these proteoglycans with collagen and/or basal laminar glycoproteins, and their GAG degradative capacities. These data should provide the means of assessing mechanisms by which the basal lamina may be assembled, and the processes that may be involved in its degradation or lack of formation upon malignant transformation.

116. STROMA IN NEOPLASTIC AND PRENEOPLASTIC MAMMARY GLAND

Bernfield, M. R., Stanford University, School of Medicine, Dept. of Pediatrics, *Stanford, California*, 94305, United States of America

The proposed study will define the role of basal lamina, its constituent glycosaminoglycan (GAG, acid mucopolysaccharide), and specific stromal tissue in mammary morphogenesis occurring post-natally and during pregnancy. The overall rationale for this research is that such a characterization will provide the basis for future studies examining whether the growth or invasiveness of mammary cancer involves abnormalities in the stromal requirement, or in the synthesis, degradation, or distribution of basal lamina GAG. This proposition is strengthened by the finding that mammary carcinoma can be induced by mammary mesenchyme to acquire differentiated elements and to organize materials tentatively identified as GAG (4), and that in human specimens basement membrane material thought to be GAG is altered in mammary carcinoma.

117. CHARACTERIZATION OF THE ISOLATED CELLS OF MAMMARY EPITHELIUM IN NORMAL AND NEOPLASTIC STATES

Tseng, M. T., University of Louisville, School of Medicine, Dept. of Anatomy, *101 W. Chestnut, Louisville, Kentucky*, 40202, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

118. MEMBRANE OF THE MAMMARY GLAND - STRUCTURE AND DYNAMICS UNDER NORMAL AND PATHOLOGICAL CONDITIONS

Pinto da Silva, P., Peixoto de Menezes, A., Parkison, C., Mather, I., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Membrane Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

OBJECTIVE: Comparative morphology of the plasmic membranes from lactating tissues and mammary carcinomas: a) Membrane events during milk fat secretion; b) transformation of endoplasmic reticulum and Golgi membrane during formation of fat droplet; c) structural transformation of the membrane envelope of the milk fat globule after secretion and during isolation procedures.

BIBLIOGRAPHIC REFERENCES: Peixoto de Menezes, A., and Pinto da Silva, P. Fat droplet formation in rat lactating mammary gland and mammary carcinomas viewed by freeze-fracture. Lab Investigation 40: 545-553, 1979.

119. EPITHELIAL-STROMAL INTERACTIONS OF BREAST CARCINOMA

Armstrong, R. M., University of California, San Francisco Campus, School of Medicine, Dept. of Anatomy, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

This proposal will apply some of the concepts of developmental biology to a study of the possible role of epithelial-stromal interactions in infiltrating ductal carcinoma of the human breast. The general approach will be to view breast carcinoma not as an isolated abnormal entity but as a series of interactions between differing tissue types beginning with alterations of cell growth patterns and progressing through invasion and metastasis. A model in vitro system for co-culturing the breast carcinoma cells with various types of 'primitive' stromas for periods of up to 10 days will be developed. The plan is to simulate laboratory conditions which will induce the carcinoma cells to display their capacities for growth, invasiveness and differentiation in a controlled environment. These properties of the cell will be examined in detail by autoradiographic and electron microscopic methods. This type of information could enhance our knowledge of the biological properties of the cancer cells in relation to surrounding tissues. In the long run, biochemical methods could be used to define more precisely the factors which may be of importance in influencing the behavior of cancer cells under various environmental conditions.

120. CELL SURFACE MODULATION IN MAMMARY NEOPLASIA

Thompson, K., Abraham, S., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, *51st & Grove Sts., Oakland, California*, 94609, United States of America

Antigenic cell surface modulation associated with malignant transformation will be investigated in order to obtain a clear distinction between the normal, preneoplastic and neoplastic mammary epithelial cells. Initial studies on the cell surface components of normal and tumor mammary epithelial cells have established that the normal mammary cell surface membrane has an additional component which can be radiolabeled with either fucose or iodine. Further research will be aimed towards characterization of the antigenic structure of normal and tumor cell surfaces. Xenogeneic antibodies have been prepared to normal mammary epithelial cell-specific antigens and to virus-associated antigens. Syngeneic antibodies to mammary tumors (from tumor-bearing animals) have also been isolated. After appropriate absorptions, these antibodies will be used in immunofluorescence studies to determine the presence and location of the respective antigens and the interrelationship with other normal cell surface antigens (i.e., H-2, Thy. 1). In addition, these specific antibodies to normal and tumor-associated antigens will be utilized in radioimmunoprecipitation experiments followed by gel electrophoresis to characterize the respective antigens. Both the qualitative and quantitative differences in cell surface structure of normal and tumor mammary epithelial cells will be defined. Preneoplastic (HAN) epithelial cells will be compared to both normal and tumor mammary epithelial cells in order to assess early events in cell surface modulation accompanying the preneoplastic state.

121. PLASMA MEMBRANE AND METASTASIS IN RAT MAMMARY CANCER

Fairbanks, G., Lewis, R. G., Worcester Foundation for Experimental Biology Inc., *222 Maple Ave., Shrewsbury, Massachusetts*, 01545, United States of America

The proposed research is a study of the relationship between mammary tumor cell surface properties and the tumorigenicity and metastasis of the cells in a syngeneic host. Several tumor cell variant subpopulations have been isolated from the transplantable 13762 rat mammary adenocarcinoma by cloning and selection in vitro. The metastatic variants will be characterized in vivo by analysis of tumor growth at subcutaneous injection sites, short-term cell survival and organ selectivity of tumor cell arrest following injection of (125I) iododeoxyuridine-labeled cells, and patterns of long-term metastatic growth. Characterization in vitro will emphasize aspects of cell adhesiveness, to be defined by measurements of adhesion to substratum, self-aggregation, and attachment to monolayers of rat fibroblasts and endothelial cells. In biochemical studies of cell surface macromolecular composition and organization, glycoproteins, proteoglycans, antigens, and lectin-binding proteins will be identified by radiolabeling, enzymatic dissection, immunoprecipitation, and specific ligand binding; these components will be partially characterized by gel electrophoresis, and their patterns of organization will be determined using protein cross-linking reagents. The kinetics and selectivity of tumor cell surface shedding will be studied in vitro. Several variants differing in metastatic activity will be examined, so that results of biochemical analysis and in vitro characterization can be correlated with patterns of mammary tumor cell behavior in vivo.

122. STUDIES OF NORMAL AND MALIGNANT HUMAN BREAST EPITHELIUM IN VITRO

Blackstein, M. E., University of Toronto, Faculty of Medicine, Dept. of Anatomy (Histology), *92 College St., Toronto, Ontario*, M5S 1A1, Canada

We have developed a cell culture system which is capable of detecting colony formation derived from single cells both from normal human breast epithelium and malignant breast epithelium. The in vitro system requires a source of serum and has an absolute requirement for exogenously added insulin and hydrocortisone. In addition, a variety of other substances have been tested, including growth factors such as EpGF, LATS and a variety of polypeptide hormones and steroid hormones. To date, none of these other substances have had significant effect (other than thyroid hormone) on colony formation from either normal or malignant epithelium.

Studies on normal breast epithelium include interaction between the variety of cells present including foam cells and non-breast epithelial cells and their necessity in colony formation. A variety of other studies, including growth and differentiation as judged by induction of protein markers such as casein and lactalbumin and lactose synthetase production are under way. Studies on epithelial cells from normal women with a strong family or genetic background of breast cancer are being undertaken to attempt to determine if the genetic susceptibility to breast cancer resides within the breast genetic information or is exogenous (endocrine abnormalities). Studies on malignant breast epithelium include the ability to induce differentiation with a variety of substances and the nature of self renewal of the colony forming unit. Suspension culture of breast tumor cells can be most readily obtained when they occur from pleural effusion material. Using this as a model, we have been able to develop a collagen matrix system in which solid breast epithelial cells from the primary tumor are dissociated and aparently undergo selective growth with suppression of non-malignant and fibroblast contaminants. Studies are now under way to determine if such a system can be adapted to test in vitro drug and hormonal sensitivities.

C. GROWTH, KINETICS AND METASTASIS OF BREAST CANCER

123. MECHANISMS OF METASTASIS

Kim, U., Park, B. H., Roswell Park Memorial Inst., Dept. of Pathology, *666 Elm St., Buffalo, New York*, 14263, United States of America

In recent years we have developed many spontaneously metastasizing rat mammary carcinomas with varying degrees of virulence. These tumors shed soluble, organ-specific antigen in rough proportion to their metastasizing capacity. Such antigen can be detected in the sera of rats as early as one week after receiving the tumor implantation, even before the graft is palpable, and its level increases as the tumor grows. Syngeneic antibodies against this antigen cannot be raised in W/Fu rats, as is the case in man in regard to such tumor-associated antigens. Utilizing this tumor system, we plan to study the mechanisms of action of this soluble cell surface antigen on the host immune system, especially the function of T- and B-lymphocytes, macrophages, and natural killer cells, both in syngeneic rats and in athymic nude mice which reject these metastasizing tumors. An attempt will also be made to learn the biochemical mechanisms of tumor cell invasiveness, antigen shedding, and metastasis by studying the activity of lysosomal enzymes, plasma membrane marker enzymes and glycosyltransferases in these tumors.

BIBLIOGRAPHIC REFERENCES: Kim, U. - Factors Influencing Metastasis of Breast Cancer. In Breast Cancer, Advances in Research and Treatment, Current Topics, Vol. 3, W. L. McGuire (Ed.), Plenum Medical Book Co., New York, pp. 1-49, 1979.

124. HUMAN SERUM INHIBITION OF MALIGNANT MAMMARY CELL GROWTH

Gaffney, E. V., Pennsylvania State University, University Park Campus, School of Science, Dept. of Microbiology & Cell Biology, *211 Whitmore Lab., University Park, Pennsylvania*, 16802, United States of America

The diagnostic and therapeutic usefulness of the initial observation that human serum inhibits the growth of malignant human mammary cells in primary cultures or established cell lines is under investigation. The studies are concerned with the isolation and characterization of the factor(s) responsible for inhibition, the procedures of serum preparation which lead to the generation of inhibitory activity, the assay of activity within populations and the macromolecular changes following exposure to human serum. Results, thus far, show that cellular DNA synthesis is severely reduced, resulting in a reversible growth arrest. This effect is observed with defibrinogenated plasma or plasma derived serum and is not affected by the presence or absence of platelet growth factors. However, inhibitory activity is observed in only a portion of donor samples. A combination of column chromatography, electrophoresis, salt precipitation and membrane filtration reveals that the activity is due to a polypeptide normally associated with serum albumin. Future research includes the development of a radioimmunassay and a study of antitumor activity.

125. HYDROXYPROLINE IN BONE METASTASIS FROM BREAST CANCER

Palmieri, G., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1030 Jefferson Ave., Memphis, Tennessee*, 38104, United States of America

In this study we plan to develop quantitative markers of collagen metabolism and bone scintiscanning for the early diagnosis and a study of the evolution and response to therapy of skeletal metastasis from breast cancer.

We hope to determine whether the simpler hydroxyproline/creatinine ratio in urine obtained during a post-absorptive period could replace the cumbersome 24h urine collections for hydroxyproline and creatinine after 2-3 days in a low gelatin diet, in patients with cancer of the breast with or without bone metastasis and in normal subjects. Also, to determine if the early (24-48h) effect to chemotherapy on the excretion of dialyzable and nondialyzable hydroxyproline has prognostic value in the long-term response to chemotherapy.

We will correlate the semiquantitative estimations of bone scans, and develop techniques for measuring hydroxylysine glycosides in order to discriminate the source of elevated excretion in patients with metastatic breast cancer (i.e., bone vs soft tissue).

126. MECHANISMS OF GROWTH, METASTASIS AND REGRESSION OF HUMAN BREAST CANCER IN NUDE MICE

Shafie, S., Liotta, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Solid mammary tumors are induced in nude mice by s.c. injections of the human breast cancer cell lines MCF-7 or ZR-75. Tumor induction is estrogen dependent, and prolactin (but not progesterone) appears to also have a stimulatory effect. The high frequency of lung, spleen and liver metastases makes this system particularly useful in the ongoing study on the mechanism of basement membrane invasiveness by tumor cells and the role of hormone both in vivo and in vitro in this process. Tumor growth proceeds even when the tumor host is ovariectomized but tumors regress when the host is treated with antiestrogens. This raised the possibility that these tumor cells continue to be estrogen dependent but have the potential of making their own hormones. We are investigating the possibility of a paraendocrine activity by these cells and their solid tumors and are in the process of designing experiments on combined endocrine and chemotherapeutic protocols that could induce an appropriate and most effective tumor regression.

127. TISSUE GROWTH FACTORS

Friesen, H. G., Shiu, R. P., University of Manitoba, Dept. of Physiology, *Winnipeg, Manitoba*, R3T 2N2, Canada

OBJECTIVES: A variety of tissue growth factors stimulate the growth of normal as well as neoplastic cells. In turn, neoplastic cells elaborate factors which stimulate growth of normal cells. This proposal seeks to: 1) Identify and characterize brain and pituitary growth factors which influence growth of normal and neoplastic cells; 2) identify and characterize growth factors in breast cancer cells which influence the growth of fibroblasts and possibly the growth of the neoplastic cells themselves.

APPROACH: Growth factors in brain, pituitary and breast cancer extracts will be purified using conventional chromatographic procedures and the mitogenic activity identified by an increase in thymidine incorporation or an increase in cell number using a variety of tissue culture techniques. After the purification of each, specific radioimmunoassays and receptor assays will be developed to be used to determine the tissue distribution, secretion and hence blood level of each growth factor. The effect of growth factors on the growth of neoplastic cells will be determined. Receptors for each growth factor will be sought in a variety of neoplastic cells.

128. METASTASIS AND INVASION BY MOUSE MAMMARY TUMORS

Nandi, S., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California*, 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

129. STUDY OF THE GROWTH KINETICS OF EPIDERMIS, SEBACEOUS GLANDS AND HUMAN MAMMARY TUMORS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Laurence, E. B., Spargo, D. J., University of London, Birkbeck College, Dept. of Zoology, *Malet St., London WC1E 7HX, England, United Kingdom*

OBJECTIVE: To determine the different cellular mechanisms involved in the response to endogenous regulators of cell proliferation of epidermal cells in: 1) short-term skin organ culture, 2) long-term cultures of pure dissociated epidermal cells and 3) the in vivo situation. A comparison will be made with sebaceous and mammary glands.

APPROACH: Mouse pinna epidermis and sebaceous glands will be studied in vivo using the technique of Thornley, Spargo and Laurence (1977, Brit. J. Derm. 97, 11023) in short-term organ culture already established (Spargo, submitted Ph. D. thesis, University of London); and in epidermal cells established through 14 generations in vitro by Dr. P.A. Riley, (Dept. of Biochemical Pathology, University College Hospital Medical School, London) with whom this work will be performed. Short-term organ cultures of human mammary glands will be used for comparison with their homologue, the sebaceous glands. The results will be obtained using standard autoradiography and scintillation counting techniques.

Analysis of the effects of time (circadian rhythm, time in culture, etc.), stress, stress hormones, beta-adrenergic agents and purified pig skin fractions on the cell kinetics will be conducted. The results should identify the existing similarities and differences between populations of epidermal cells in the three systems. The nucleotide pools and enzyme activity e.g., thymidine kinase, ornithine decarboxylase and adenylate cyclase will be analyzed, and where possible, these measurements will be related to the cell cycle events.

It is proposed to ascertain whether the 72-81 percent ethanol fraction of pig skin which is known to contain sebaceous gland chalone also inhibits the cell proliferation of its homologue, the mammary gland. It is necessary to test this in vitro since hormonal effects mask the activity of normal tissue extracts in vivo.

Lastly it is proposed to use these systems to study skin carcinogens e.g., application of dimethyl benzanthracene and phorbol esters at various stages of the cell cycle and in different physiological conditions.

130. GROWTH RATE OF TRANSPLANTABLE MAMMARY ADENOCARCINOMA (MICE)

Abraham, S., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, *51st & Grove Sts., Oakland, California*, 94609, United States of America

This is part of a broader project. A summary of this subproject is not available.

131. HYDROXYPROLINE IN BONE METASTASIS FROM BREAST CANCER (HUMAN)

Palmieri, G. M., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Medicine, *62 S. Dunlap St., Memphis, Tennessee*, 38103, United States of America

This is part of a broader project. A summary of this subproject is not available.

132. CELL PROLIFERATION IN MOUSE MAMMARY GLANDS

Lee, A. E., Imperial Cancer Research Fund, Hormone Physiology Laboratory, *Lincoln's Inn Fields, c/o St. Bartholomew's Hospital, London WC2A 3PX, England, United Kingdom*

It has been shown that in BR6 mice, the presence of a mammary tumor virus (MTV) affects the response of the mammary glands of ovariectomized animals to exogenous estrogen and progesterone (ICRF Scientific Report 1977 - report 97). Cell proliferation as indicated by the mitotic index (M.I.) and 3H-thymidine-labelling index (L.I.) has been compared throughout the estrous cycle and during pregnancy in MTV-carrying mice, with an expected tumor incidence in breeding females of 94 percent, and MTV-free mice which do not develop tumors.

The stage of the cycle was determined by inspection of vaginal smears. Mammary epithelial M.I. and L.I. were maximal at estrus in both groups. An overall difference between the groups was not significant in this experiment, but the MTV-free mice appeared to have decreased proliferative activity, especially during di-estrus and pro-estrus.

The pattern of cell proliferation during first pregnancies was similar in both groups, with peaks of activity on days 4-5 and 12 (the finding of a vaginal plug was designated day 1 of pregnancy). There was no apparent difference between the levels of activity reached by the two groups. Since tumors do not usually appear in MTV-carrying females until after several pregnancies, it may be that the influence of MTV is not a critical factor during the earlier pregnancies.

133. BIOCHEMICAL CHARACTERIZATION OF RAT MAMMARY TUMOR CELL GROWTH FACTORS

Sirbasku, D. A., University of Texas, Health Science Center at Houston, School of Medicine, Dept. of Biochemistry & Molecular Biology, *Box 20036, Houston, Texas*, 77025, United States of America

We are studying the role of growth factors in the regulation of the proliferation of rat mammary tumor cells in vivo and in vitro. Our working hypothesis is that in vivo estrogens promote growth of some types of hormone responsive tumor cells via an indirect mechanism of induction and/or release of polypeptide growth factors which then act as the major mitogens. We have shown that estrogen-inducible mammary growth activities exist in the uterus and kidney and our hypothesis is that these activities assume this mediator or 'estromedin' role in vivo. The model for such an estrogen-controlled growth mechanism may be summarized as ESTROGENS - UTERUS AND KIDNEY - GROWTH FACTORS - MAMMARY TUMOR GROWTH. Our previous studies have shown: firstly, the existence of estrogen-related mammary tumor growth factor activities in uterus and kidney; secondly, that the uterine derived activity was relatively specific for cells which formed estrogen-responsive tumors; thirdly, that the uterine derived activity possesses polypeptide characteristics; and fourthly, that estrogens cause accumulation of mammary growth factor activity in extracellular secretions of uterine luminal fluid. Most recently, we have shown that 125I-labeled proteins injected into the uterine luminal fluid can reach the general circulation in an undegraded state. Taken together, these observations all suggest that the estromedin mechanism is possible in vivo. While the evidence accumulated thus far is consistent with our estromedin hypothesis, further key supporting data was obtained by identifying a uterine-like growth factor activity in association with mammary tumor cells growing in vivo.

The studies outlined in this continuation project are a direct extension and further refinement of our earlier observations. We propose to continue purification of the uterine and kidney derived activities, develop radioimmunoassay methods of growth factor measurement, and to continue studies of the properties of the growth activity in estrogen-accumulated uterine luminal fluid, plasma, and in extracts of the estrogen-responsive MTW9/PL tumors growing in vivo. Also, we will continue cloning the MTW9/PL cell line to obtain potentially useful submembers of the population.

134. GROWTH CONTROL OF MAMMARY CELLS AND BREAST CANCER

Dulbecco, R., Salk Inst. for Biological Studies, *10010 N. Torry Pines Blvd., P.O. Box 1809, San Diego, California*, 92112, United States of America

This is part of a broader project. A summary of this subproject is not available.

135. REGULATION OF MAMMARY GLAND GROWTH AND REGRESSION

Tang, F. Y., University of Rochester, School of Medicine & Dentistry, Dept. of Obstetrics & Gynecology, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

Growth of tumors of the mammary gland can be arrested by ovariectomy in most but not all 7,12-dimethylbenz(alpha)anthracene (DMBA)-induced rat mammary adenocarcinomas. Actual tumor regression in these animals involves production of cyclic adenosine-3',5'-monophosphate (cAMP) and stimulation of cAMP-dependent protein kinase. It has been proposed that a cytoplasmic receptor-kinase (RC) complex is translocated to the nucleus during the initial events leading to tumor regression.

Data from this laboratory have shown that DMBA-induced rat mammary tumors which fail to regress after ovariectomy exhibit an excess of free catalytic (C) subunit activity in their cytosols which is not present in the cytosols of either mammary gland or regressing mammary tumor. The mammary tumors which fail to regress are classified as ovarian hormone independent and exhibit free regulatory (R) subunit activity and a 10-fold lower affinity for cAMP binding than either mammary gland or hormone-dependent (regressing) mammary tumor. Moreover, the hormone-independent mammary tumors fail to accumulate R and C subunit activity in their nuclei. The presence of free R and C subunits in hormone-independent mammary tumor cytosols suggests a failure of an R and C subunit interaction, presumably due to a defective R subunit, which may be necessary for achieving nuclear translocation of the receptor-kinase (RC) complex. In this proposal, we wish to investigate the phenomenon of cytoplasmic 'activation' of the RC complex in regressing rat mammary tumors and determine whether or not cytoplasmic activation of an RC complex may be associated with nuclear accumulation of R and C subunits. Investigation of the occurrence of cytoplasmic receptor activation may provide evidence which may or may not support the concept of nuclear translocation of the receptor-kinase (RC) complex.

136. HORMONE-DEPENDENCE AND AUTONOMY IN TUMOR GROWTH

Stanley, E. R., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Microbiology & Immunology/Cell Biology, *1300 Morris Park Ave., Bronx, New York*, 10461, United States of America

We have recently developed an experimental system in which the nature of the transition between hormone-dependent and autonomous states in cells from an androgen-dependent mouse mammary adenocarcinoma (Shionogi Carcinoma 115) can be examined using genetic and biochemical approaches. This system involves the use of cloned, cultured androgen-dependent and autonomous cell lines and culture conditions under which growth of dependent cells is dependent on the presence of added androgen. It is possible to determine the proportion of dependent and autonomous cells in mixed populations and to grow the cell lines as tumors in mice. We propose to use this system to analyze the nature of the transition between dependence and autonomy and to study the mechanism of androgen regulated cell proliferation.

137. THE ROLE OF THYROID HORMONES IN GROWTH AND DIFFERENTIATION OF MOUSE MAMMARY GLANDS

Vonderhaar, B. K., Bhattacharya, A., Greco, A. E., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project is to understand the role of thyroid hormone-prolactin interactions in the normal growth and differentiation of the mammary gland and the development of mammary tumors. Studies include: 1) examination of development and growth of 'spontaneous' mammary tumors in primiparous mice in various thyroid states; 2) evaluation of the role of thyroid hormones in induction of mammary carcinomas in rat by N-nitrosomethylurea; 3) examination of the regulation of prolactin receptors in mammary tissue by thyroid hormones in vivo and in vitro; 4) determination of the effects of altered membrane lipid composition on prolactin binding; 5) characterization of thyroid hormone binding to mammary tissue using biologically active fluorescent thyroid hormone derivatives.

BIBLIOGRAPHIC REFERENCES: Vonderhaar, B.K., and Greco, A.E.: Lobulo-alveolar development of mouse mammary glands is regulated by thyroid hormones. Endorcinology 104: 409-418, 1979. Vonderhaar, B.K.: Lactose synthetase activity in mouse mammary glands is controlled by thyroid hormones. J. Cell Biol. in press 1979.

138. NORMAL AND NEOPLASTIC DEVELOPMENT OF THE MAMMARY GLAND

Dulbecco, R., Unger, M., Battifora, H., Bennett, D., Salk Inst. for Biological Studies, Dulbecco Laboratory, *10010 N. Torry Pines Blvd., P.O. Box 1809, San Diego, California*, 92112, United States of America

The analysis of cell types in rat mammary gland based on their growth response to the phases of the estrus cycle will be completed. An analysis of lesions induced by nitrosomethylurea in the rat mammary gland will be carried out, and the cell types involved will be determined. The specificity of monoclonal antibodies prepared to rat and human mammary cells will be studied, in terms both of the cell types to which they bind in vivo and of the cell surface proteins they immunoprecipitate in cultures. New monoclonal antibodies to human mammary cells, both normal and neoplastic, will be isolated. The role of the Thy-1 antigen in the differentiation of rat mammary cells will be studied further. The mechanism by which lipids (fatty acids, lysolecithins) induce dome formation in cultures of rat mammary cells will be pursued.

BIBLIOGRAPHIC REFERENCES: R. Dulbecco, M. Bologna, and M. Unger. Differentiation of a rat mammary cell line in vitro. Proc. Natl. Acad. Sci. USA, 76, 1256-1260 (1979). R. Dulbecco, M. Bologna, and M. Unger. Role of Thy-1 antigen in the in vitro differentiation of a rat mammary cell line. Proc. Natl. Acad. Sci. USA, 76, 1848-1852 (1979).

139. CONTROL MECHANISMS OF MAMMALIAN CELL GROWTH

Kano Sueoka, T., University of Colorado, Boulder Campus, School of Arts & Sciences, Dept. of Molecular Cellular & Developmental Biology, *1200 University Ave., Boulder, Colorado*, 80302, United States of America

We propose to study the mechanisms which regulate the multiplication of hormone-dependent rat mammary carcinoma, in particular to identify the hormones and factors which tumor cells require for growth using in vivo and in vitro systems, and to examine how these factors and hormones regulate growth. We will use hormone-dependent (HD) and hormone-independent (HI) tumors which originated from a rat (AxC) mammary carcinoma and clonal cell lines isolated from these tumors which possess specific growth requirements in vitro. We will also use primary culture of normal rat mammary gland. The HD cell line requires for growth MGF (identified to be phosphoethanolamine) in calf serum-supplemented medium or phosphoethanolamine and a group of hormones in serum-deprived medium.

We will study the mode of action of phosphoethanolamine first by determining whether phosphoethanolamine acts like a hormone or a nutrient. The synergistic effect with other hormones will also be investigated. We will further determine the functional specificity of phosphoethanolamine. This study should show us whether phosphoethanolamine is effective only on certain mammary tumor cells or on normal mammary tissue as well, and whether it can also act on cells from other tissues and organs.

The HD tumors produce significant amounts of alpha-lactalbumin, a component of lactose synthetase. In addition, the tumors and their clonal cell lines in culture produce a large amount of fat. Once we determine whether the fat synthesized is milk specific, we intend to analyze fat and alpha-lactalbumin

(and possibly casein) in both tumors (in vivo and primary culture) and cell lines under varied hormonal environments, in order to understand how growth and differentiation capabilities of the cells are regulated.

140. EFFECT OF MAMMARY CANCER CELLS ON VASCULAR ENDOTHELIUM

Lindquist, R. R., University of Connecticut, Health Center, School of Medicine, Dept. of Pathology, *Farmington Ave., Farmington, Connecticut*, 06032, United States of America

OBJECTIVES: Elucidation of the mechanisms involved in the development of tumor metastases, because rational methods of preventing and/or containing tumor metastases can only be developed after the pathogenesis of metastases is known. Elucidation of the cancer cell-endothelium interaction and the effect cancer cells have on the endothelium. To obtain greater information on the cancer cell-endothelium interaction, the following will be undertaken: morphologic examination by time-lapse cinemicroscopy and scanning electron microscopy of cancer cell-endothelium interaction in vitro; qualification of the number and strength of cancer cell-endothelium interaction and features that influence the interaction; and determination of the effect cancer cells have on endothelium.

141. CHANGING SENSITIVITY TO CARCINOGENESIS OF MAMMARY GLAND

Sinha, D. K., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

Study the prepubertal, pubertal, and postpubertal growth pattern of the mammary gland in relation to carcinogenesis. Investigate the induction of pubertal-type growth in 'aging' mammary glands, and produce tumors in them by a chemical carcinogen, and induction of mammary tissue aging in immature rats to study the possibility of inhibition of mammary carcinogenesis. Study the biological and biochemical characteristis of mammary cells from nulliparous, pregnant, lactating, and multiparous rats in relation to their susceptibility or refractoriness to carcinogens.

142. REGIONAL DIFFERENCES IN GROWTH OF BREAST TUMORS OF SPONTANEOUS ORIGIN IN THE RAT

Matos, A. N., Martinez, M. R., Universidad de Oriente, Escuela de Medicina, Ciencias Fisiologicas - Seccion Farmacologia, *Apartado 356, Ciudad Bolivar, Caracas, Venezuela*

OBJECTIVES: To study the mechanism of regression of the Sp4 tumor inoculated in the right anterosuperior region of the thorax.

APPROACH: To study the possible role of hormones and the immune system.

PROGRESS: Studies which we performed in the University of Nottingham, England, in 1978 indicate that spontaneous mammary adenocarcinoma in Wistar/Not. rats (Sp4), by intramammary injection, demonstrate different growth characteristics according to the site of the intramammary injection. The adenocarcinoma grows continually, leading to death, if injected in the right mamillothoracicoabdominal region; it regresses spontaneously if injected in the superior mamilla of the same side. We are currently reproducing these results in our Department with the same congenital strain of Wistar/Not. provided from England and maintained by crossing among offspring of the same litter.

143. CELL PROLIFERATION AND LOSS KINETICS IN HUMAN TUMORS AND LIMITING NORMAL TISSUES

Hamilton, E. A., Berry, R. J., University of London, Middlesex Hospital Medical School, Dept. of Oncology, *Riding House St., London W1P 7LD, England, United Kingdom*

OBJECTIVE: The measurement of cell proliferation and loss in human breast tumors in situ before and during treatment.

APPROACH: Iso-transplanted murine mammary carcinoma will be used to develop methods of measuring the cell kinetics of tumors in situ. The results of such methods will be compared with the cell kinetics parameters of the model tumors obtained by conventional techniques involving isotope tracer administration and sacrifice of the animal. Measurements will then be made, with the methods developed, of human breast tumors in situ. The techniques to be used are: 1) Serial sampling to study mitotic accumulation after an injection of vincristine. This technique will be used to analyze both untreated and irradiated tumors. When the results can be interpreted in terms of the tumor cell kinetics, serial biopsies from patients undergoing vincristine therapy will be analyzed. 2) Injection and external counting of radioactive iododeoxyuridine (IUdR). Techniques will be developed for local injection of this nucleotide into or around the tumor. IUdR will then be administered locally to breast cancer patients and the tumor will be scanned regularly with a gamma camera. We hope to estimate the proportion of actively proliferating cells and their location in the tumor. Loss of cells from the tumor will also be estimated.

PROGRESS: This is a newly funded project.

144. BREAST CANCER KINETICS STUDIES (HUMAN)

Bodey, G. P., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Developmental Therapeutics, *c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

This is part of a broader project. A summary of this subproject is not available.

145. PLASMA MEMBRANE AND METASTASIS IN RAT MAMMARY CANCER

Fairbanks, G., Lewis, R. G., Worcester Foundation for Experimental Biology Inc., *222 Maple Ave., Shrewsbury, Massachusetts*, 01545, United States of America

The summary for this project is under entry no. 121.

146. IMMUNE MECHANISMS INVOLVED IN TUMOR METASTASIS

Roholt, O. A., Kim, U., Fuji, H., Ghosh, S. K., Roswell Park Memorial Inst., Dept. of Immunology Research, *666 Elm St., Buffalo, New York*, 14263, United States of America

Our studies are intended to clarify some of the mechanisms that operate in tumor metastasis. We are investigating the role of membrane-associated components of tumors, including tumor associated antigens, tumor specific antigens and tumor specific transplantation antigens. We are also investigating the role of specific anti-tumor antibodies and of tumor specific immune complexes. We are studying the interactions between these membrane-associated and humoral components and the various immunocompetent cells involved in the immune response to tumors. Such studies have the ultimate objective of providing new information that can be applied to the prevention or cure of metastatic disease.

For these purposes we are employing a relevant animal model -chemically induced, transplantable rat mammary carcinomas, both metastatic and nonmetastatic. In initial studies we have identified and quantitated a common organ specific tumor associated antigen, apparently characteristic of our metastatic tumors. This antigen is shed by the tumors and may be functionally involved in the metastatic process.

The components of interest are being identified and isolated by specific methods which include the use of immunoadsorbents, lectin affinity adsorbents and xenogeneic antibodies specific for the various membrane-associated components. Sensitive and specific radioimmunoassays are being developed to quantitate these components. Similar sensitive assays are being developed to both detect and quantitate specific anti-tumor antibodies and the specific immune complexes.

A prime objective of these studies is to determine how, and to what extent, the various immunologically defined components are involved in the metastatic process. A second objective is to use the specific analytical procedures we are developing in the animal system as guides in developing procedures for early detection of human tumors.

147. HORMONAL CONTROL OF METASTASIS

McGrath, C. M., Soule, H. D., White, A., Michigan Cancer Foundation, Dept. of Biology, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

The summary for this project is under entry no. 58.

D. GENETIC STUDIES OF BREAST CANCER

148. ALTERED GENOME EXPRESSION IN PRENEOPLASIA AND NEOPLASIA

Abraham, S., Hillyard, L., Miyamoto, M., Hansen, F., Childrens Hospital Medical Center of Northern California, Bruce Lyon

Memorial Research Laboratory, *51st & Grove Sts., Oakland, California,* 94609, United States of America

In order to pursue our overall objective of finding specific means of altering the expression of the transformed genome of mammary epithelial cells, whether they are preneoplastic or neoplastic, we have: a) studied the influence of dietary lipids on the growth rate of transplantable mammary adenocarcinomas in both virus-infected and uninfected mouse strains as well as in rats, b) performed membrane studies with isolated cells from both normal and tumor murine mammary tissues, and c) developed cell surface markers which can distinguish epithelial cells, adipose cells, and fibrocytes from each other when grown in culture.

Our results suggest that the tumor promoting effect of dietary fat: a) is caused by cis, cis-linoleate; b) varies quantitatively with species and strain of animal; c) cannot be accounted for by an increased neoplastic cell proliferation and therefore, d) may be mediated through the cellular immune system.

BIBLIOGRAPHIC REFERENCES: 'Relationship Between the Multiple Molecular Forms of Glucose 6-Phosphate dehydrogenase and Glutathione in Normal, Preneoplastic and Neoplastic Mouse Mammary Tissue,' by Russell Hilf, Regina Ickowicz, J.C. Bartley, and S. Abraham, Cancer Biochem. Biophys. 2:191-197, 1978. 'Triglyceride Synthesis of Dihydroxyacetone Phosphate and Palmitate by Microsomes from Mammary Glands of Lactating Mice,' by G. Ananda Rao and S. Abraham, Lipids 13:95-98, 1978.

149. GENETIC EPIDEMIOLOGY AND NATURAL HISTORY OF BREAST CANCER

Petrakis, N. L., King, E. B., Maack, C. A., Kelly, P. T., Ernster, V. L., Sacks, S. T., King, M. C., University of California, San Francisco Campus, School of Medicine, Dept. of Epidemiology & International Health, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

Epidemiological and experimental studies are under way to investigate host, agent and environmental factors associated with breast cancer. A working model has been developed that relates epidemiological evidence linking reproductive experience and the risk of breast cancer to our findings regarding breast secretory activity in different groups and evidence of secretion of exogenous substances through the breast. Current research focuses on genetic markers associated with breast cancer, cytopathologic characteristics of normal and malignant breast tissue, metabolic activation of mutagenic agents by breast tissue, and secretion of potentially carcinogenic substances in breast fluid. In addition, a study of women's beliefs and health practices concerning breast cancer is under way.

150. CHROMOSOMAL INDICATORS OF MALIGNANCY

Gebhart, E., Pfeiffer, R. A., Friedrich Alexander Universitat zu Erlangen Nurenberg, Institut fur Humangenetik und Anthropologie, *Bismarckstrasse 10, D852 Erlangen, Federal Republic of Germany*

OBJECTIVE: By chromosome studies in solid tumors, as well as long-term cell cultures derived from solid tumors and from precancerous lesions, specific patterns of chromosome damage of malignant deterioration are investigated. Comparison of karyotypes from precancerous lesions and malignant tumors as well as from tumors and metastases and from direct preparations and derived cell cultures is expected to yield evidence on karyotype evolution in malignancy.

APPROACH: The studies are performed on excision material from surgical specimens of solid breast tumors as well as from precancerous lesions of the mouth epithelium. Material from other areas (e.g., colorectal carcinoma, neoplasias of the mouth and nasopharynx) will also be included in these studies.

PROGRESS: Since the studies were started in 1978 so far only technical problems of tumor cell culture and chromosome preparation from solid tumors have been handled. First observations on the existence of 'double minutes' also in solid breast tumors (not only in cultured cell lines) have been made. Further progress in evaluation of specific cytogenetic changes related to malignancy is expected as a result of the work of the next three years.

151. ASSAY FOR GENETIC DAMAGE TO MAMMARY GLAND CELLS

Hart, R. W., Ohio State University, Columbus Campus, School of Medicine, Dept. of Radiology, *370 W. 9th Ave., Columbus, Ohio,* 43212, United States of America

OBJECTIVE: Devise or design an assay for detecting genetic damage or changes that may increase the rate of damage in mammary cells. General possibilities for assessing genetic damage might be the enumeration of cells with mutant phenotypes, determination of induced damage to DNA of individual cells or of populations of cells, or the enumeration of cells with evidence of mitotic disturbances. Such an assay would then be used to study the effects of age, diet, hormones, carcinogens, etc., on the incidence of genetic damage.

152. GENETIC STUDIES OF BREAST CANCER

Anderson, D. E., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Medical Genetics, *c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

The purpose of this study is to identify patients with hereditary types of breast cancer. Patients with family histories of the disease are selected for study; controls are selected from the same patient population but have family histories of neoplasms other than breast. Both groups are studied simultaneously with similar methodological approaches. Family and medical histories are obtained by questionnaires; all reported tumors are verified by a medical, hospital, pathological and/or vital statistics report. Patients are classified according to age at clinical onset, single or multiple primaries, type of family history and associated familial neoplasms. Patients were classified according to family history of breast cancer at time of ascertainment; families in which patients had an affected mother exhibited lifetime probabilities of 30 percent. When affected mothers and patients were further categorized according to age at diagnosis and laterality, families in which both patient and affected mother had premenopausal onset were characterized by a lifetime probability of 40 percent. When one or the other or a close relative had bilateral disease the probability was 34 percent. Unilateral or postmenopausal disease in both was associated with a 20 percent probability. Fifty percent probability was obtained when both had premenopausal disease and one or the other or a close relative had bilateral disease, compared with a probability of 13 percent when the disease in both was unilateral and postmenopausal. A family history of breast cancer in the sister of a patient was associated with lifetime probabilities little different from those expected for women from the general population (7 percent). The only exception was when the patient and her affected sister both had premenopausal disease and one or the other or a close relative had bilateral disease (lifetime probability 50 percent).

153. REGULATION OF GENE EXPRESSION IN MOUSE MAMMARY CANCER

Socher, S. H., Baylor College of Medicine, School of Medicine, Dept. of Cell Biology, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

OBJECTIVES: Analyze the regulation of gene expression using MMTV-RNA and casein mRNA as specific markers of gene function in normal, preneoplastic, and neoplastic mouse mammary tissues. Quantitate levels of casein mRNA and MMTV-RNA sequences using selective hybridization probes in RHA extracts isolated from the same tissues. Study mechanisms by which chromatin-associated molecules modulate gene transcription in mammary tissues. Utilize the mammary gland organ culture system to analyze hormone interactions that modulate MMTV and casein gene expression.

E. PATHOGENESIS AND HISTOLOGIC STUDIES OF BREAST CANCER

154. ASSOCIATION BETWEEN BENIGN AND MALIGNANT BREAST LESIONS

Soini, I. H., Aine, R., Lauslahti, K., Hakama, M., Tampere Yliopisto, Dept. of Public Health, *Vuolteenkatu 11, 33100 Tampere 10, Finland*

The objective of this study is to demonstrate a possible relationship between benign and malignant breast lesions using risk factor analysis of hormonal and reproductive data.

Epidemiological data were gathered from 600 women operated on for benign breast lesions between 1974 and 1977. The risk factors of benign and malignant lesions should also be related if there is a causal association between benign and malignant lesions or if they are associated without the transition of a benign lesion to breast cancer.

155. INCIDENCE AND HISTOLOGICAL TYPES OF BREAST CANCER IN ISFAHAN, IRAN

Emany, H., Monajemian, S., Rezazadeh, A., University of Isfahan, School of Medicine, Dept. of Pathology, *Hezargerib, Isfahan, Iran*

OBJECTIVE: As there is no cancer registry in the region, the compiled files of the Department of Pathology over the past 30 years are used to estimate the incidence of cancer in the region, especially cancer of the breast.

APPROACH: Review of the pathological reports and sections over the past 30 years in the Dept. of Pathology, Isfahan University, Med. Sch.

PROGRESS: So far 15000 sections and reports have been reviewed.

156. HISTOLOGICAL, CYTOLOGICAL AND HISTOCHEMICAL ALTERATIONS IN HUMAN MAMMARY CANCER

De Kastner, M. R., Schettino, A. M., Instituto Nacional do Cancer, *Caixa Postal 1675-ZC-00, 20000 Rio de Janeiro, R.J., Brazil*

The purpose of this study is to analyze in detail the pathology of breast cancer. Particular attention is directed at: 1) Evaluating the morphology of the local cellular reaction in the breast and in the axillary lymph nodes. 2) Attempting the detection of tumor-associated antigens and steroid hormone receptors. 3) Searching for distinctive patterns helpful in differentiating histologic types of mammary cancer. 4) Determining the existence of an additional histologic index reflecting the degree of aggressiveness or a valuable prognostic meaning in breast cancer.

157. PROLIFERATIVE LESIONS OF THE FEMALE BREAST - RETROSPECTIVE AND PROSPECTIVE STUDIES

Venekamp, H. W., Hampe, J. F., Nederlands Kankerinstituut, *106-108 Sarphatistraat, Amsterdam, Netherlands*

OBJECTIVE: Investigation of the relationship between apparently benign proliferative lesions of the female breast and subsequent appearing of infiltrating mammary carcinoma in ipsi- and heterolateral breast.

RETROSPECTIVE STUDY: Comparison of slides of benign proliferative lesions of women who developed mammary carcinoma afterwards with women (age-matched) who did not develop breast cancer after at least ten years of observation.

PROSPECTIVE STUDY: Study in a consecutive series of 1,000 women who were operated for apparently benign proliferative lesions. Follow-up period will be at least ten years.

APPROACH: Clinical examination; epidemiology; morbid histology; androgene metabolism; hormone receptor contents of the tissue; and cytophotometric procedures.

158. ANALYSIS OF TRANSFORMATION PROCESSES IN MAMMARY TISSUE (MICE, RATS)

Nandi, S., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California*, 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

159. CELL CONTACTS IN NORMAL AND ABNORMAL MAMMARY GLAND (MICE)

Pitelka, D. R., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California*, 94720, United States of America

This is part of a broader project. A summary of this subproject is not available.

160. EARLY BREAST CANCER - PATHOLOGY AND PROGNOSIS OF BREAST CANCER DEVELOPING IN PATIENTS TAKING ORAL CONTRACEPTIVES

Hayward, J. L., Millis, R., Matthews, P. N., Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, *St. Thomas St., London SE1, England, United Kingdom*

An analysis has been carried out on all patients with breast cancer who have taken oral contraceptives at any time in the past. Clinical and histological features and prognosis have been compared with those in matched controls. A significantly higher number of the control patients had larger tumors and more extensive axillary node involvement than was found in the study group. Furthermore, a significantly greater number of patients in the study group had close relatives (mother or sister) who had had breast cancer than in the control group but the difference was not significant. These findings tended to reinforce those which had been reported in an earlier study on patients who had been taking the contraceptive pill during the year prior to diagnosis.

161. RISK ASSOCIATED WITH CARCINOMA IN SITU AND PRECANCEROUS MAMMARY HYPERPLASIAS

Page, D. L., Rogers, L. W., DuPont, W., Federspiel, C., Landenberger, M., Vanderbilt University, School of Medicine, Dept. of Pathology, *21st Ave. S. & Garland, Nashville, Tennessee*, 37232, United States of America

The investigators are assembling, reviewing and classifying slides from breast biopsies of 4,000 women, performed 10 or more years ago and diagnosed as benign breast disease at that time. All material is being classified as to the component parts of cystic disease present, using a modification of the generally accepted categories of histological alteration included under the term 'cystic disease.' Correlation studies of the subsequent development of carcinoma, the component types of cystic disease (including papillary and lobular proliferative lesions) and various historical features of each patient will be undertaken. The final goal of this study will be to assign a predictive index to specific histologic changes permitting assignment of relative risk to individual patients for the likelihood of the development of carcinoma. Results to date after analysis of 1,000 women with biopsies performed eighteen or more years ago show that women lacking epithelial proliferative changes have the same cancer risk as the non-biopsied population at large. Various proliferative lesions usually termed 'ductal' type elevate cancer risk over twice that of the age-controlled population when identified by biopsy after age 45. Atypical lobular hyperplasia identified at biopsy elevates cancer risk to six times that of the general population.

162. THE BIOLOGY OF RAT MAMMARY HYPERPLASIAS

Rivera, E. M., Raghavan, S. V., Michigan State University, School of Natural Sciences, Dept. of Zoology, *243 Natural Resources Bldg., East Lansing, Michigan*, 48824, United States of America

The overall objective is to characterize several types of hyperplastic lesions induced by chemical carcinogen in vivo and in vitro or arising spontaneously in untreated mammary glands of old female rats. Tumors will be characterized according to the mammary tissue of origin. Attempts will be made to enhance or inhibit tumor transformation in primary and transplantable mammary hyperplasias. The methodology will include mammary fat pad transplantation, organ culture, hormonal manipulation, and morphology.

163. THE ANGLO-JAPANESE TRIAL - PATHOLOGY OF BREAST TUMORS IN GREAT BRITIAN AND JAPAN

Hayward, J. L., Bulbrook, R. D., Kumaoka, S., Friedell, G. H., Soto, H., Templeton, S., Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, *St. Thomas St., London SE1, England, United Kingdom*

It was noted (ICRF Scientific Report 1976 - Report 264) that Japanese patients with early breast cancer have a much higher incidence of sinus histiocytosis in uninvolved axillary lymph nodes than similar patients in Britain.

An attempt is now being made to compare urine and blood endocrine levels with sinus histiocytosis, in British and Japanese patients with breast cancer. Histological sections from the primary tumor and axillary lymph nodes are being obtained from those patients from whom urine and blood specimens were

collected for the original endocrine comparison between the two races. These sections have all been mounted and stained identically in the Breast Cancer Unit, and sent to St. Vincent Hospital, Massachusetts where they are being reviewed independently by three pathologists.

164. PATHOLOGY OF NORMAL AXILLARY LYMPH NODES FROM WOMEN IN GREAT BRITAIN AND JAPAN WHO DIED FROM CAUSES OTHER THAN BREAST CANCER (ABBREV)

Hayward, J. L., Millis, R., Bulbrook, R. D., Friedell, G. H., Ide, Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, *St. Thomas St., London SE1, England, United Kingdom*

Comparative studies of the pathology of breast tumors and axillary lymph nodes from patients in Great Britain and Japan have shown that Japanese patients with early breast cancer have a higher incidence of sinus histiocytosis in the uninvolved axillary lymph nodes than patients in Great Britain. The incidence of sinus histiocytosis in axillary lymph nodes from British and Japanese women without breast cancer is now being investigated. In Great Britain lymph nodes obtained from coroner's post mortems performed on women who die suddenly without evidence of any malignant disease are being collected so that the degree of sinus histiocytosis can be assessed in histological sections. Similar nodes are being collected in Japan.

165. PATHOLOGY OF BREAST TUMORS IN THE GUERNSEY PROSPECTIVE TRIAL

Millis, R., Hayward, J. L., Bulbrook, R. D., Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, *St. Thomas St., London SE1, England, United Kingdom*

Review of breast cancers occurring in women who took part in the Guernsey prospective trial continues. Histological sections from all tumors developing in these women are examined and features which may be of prognostic significance evaluated. Particular note is made of the degree of inflammatory reaction, consisting mainly of lymphocytes and plasma cells, around and within the tumor, as preliminary studies have indicated that the degree of cellular reaction may be correlated with the levels of urinary aetiocholanolone in these women.

166. PREVENTION OF MAMMARY PRENEOPLASTIC LESIONS

Welsch, C. W., Michigan State University, School of Veterinary Medicine, Dept. of Anatomy, *New Administration Bldg., East Lansing, Michigan,* 48823, United States of America

OBJECTIVES: To determine if chronic suppression of pituitary prolactin will prevent or inhibit the early development of any one, more than one, or all of these types of dysplasias and as a consequence prevent or substantially reduce the incidence of mammary neoplasias. The rodents will be treated daily with the prolactin-suppressing drug 2-bromo-a-ergo-cryptine (CB-154) after the spontaneous development of, or the induction of, these mammary gland dysplasias. To determine if chronic suppression of pituitary prolactin will prevent or inhibit the progression of any one, more than one, or all of these types of dysplasias to neoplasias and as a consequence prevent or substantially reduce the incidence of palpable mammary neoplastic lesions.

167. SMALL CELL IN SITU LESIONS OF THE FEMALE BREAST

Toker, C., Goldberg, J., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Pathology, *655 W. Baltimore St., Baltimore, Maryland,* 21201, United States of America

Small cell lesions of the female breast (lobular carcinoma in situ) are found in some 8% of breast biopsies taken for benign conditions. The long-term behavior of the lesion still awaits elucidation. Current evidence would suggest that some 20% of patients with this lesion will develop an invasive carcinoma in either the ipsilateral or the contralateral breast when followed over a 20-year period. Most of these invasive tumors will appear within 15 years after detection of the initial small cell lesion. What remains to be established is whether any relationship exists between the dimension of the original small cell lesion and the incidence of subsequent invasive carcinoma. Although our own series includes some 100 patients, and although the number is being augmented, resolution of this problem requires a much larger number, probably thousands. This will require a collaborative effort of national or international dimensions. Such an effort will require a major investment of time and money, but it will provide both prognostic and demographic, as well as, possibly, aetiological data that would adequately justify the expense.

168. QUANTITATIVE STUDIES OF HUMAN BREAST PATHOLOGY

Wellings, S. R., University of California, Davis Campus, School of Medicine, Dept. of Pathology, *Davis, California,* 95616, United States of America

Our purpose is to quantify mammary epithelial lesions in large numbers of biopsy size samples of human breast tissue using our own histological grading methods and to correlate the findings with risk as determined by other histological methods, by mammography, and by the actual appearance of cancers in the patients studied.

Differential counts of different kinds of lesions are determined, and these are scored according to severity and amount of atypia, which can be further related to area of tissue on the slides.

BIBLIOGRAPHIC REFERENCES: Wellings, S.R., H.M. Jensen, and J.D. Rice. Preneoplastic lesions in the human breast. Recent Results in Cancer Research, 1978, in press. Wellings, S.R. and J.N. Wolfe. Correlation of pathology with xeromammographic images. Radiology, 1978, in press.

169. HISTOLOGIC TYPING OF BREAST CANCER - A COMPARISON OF THREE CLASSIFICATIONS

Stalsberg, H., Hartveit, F., Haugen, O. A., Skaug, V., Universitetet i Tromso, Inst. of Medical Biology, Morphology Unit, *P.O. Box 977, 9001 Tromso 2, Norway*

The WHO Histological Typing of Breast Tumors, the histological classification of the National Surgical Adjuvant Breast Project, and a modification of the classification of the Pathology Working Group of the Breast Cancer Task Force of the NIH are compared with regard to intra-and inter-observer reproducibility, consistency, and their ability to distinguish entities of reasonable size and different age distribution, stage distribution, and survival. Based on listings from the Cancer Registry of Norway, histologic material from all cases of breast cancer histologically diagnosed in Norway during 1970 have been collected, relabelled and distributed at random to four pathologists for classification according to all three classifications. In a second round, the sets of slides were rotated among the pathologists, new slides added for reproducibility studies, and each set reclassified by a second observer. On purpose, the observers relied solely on printed material in their interpretation and use of the classification criteria, in order to test the application of the classifications as they appear in the literature.

170. PATHOGENESIS IN THE MAMMARY GLANDS (RATS)

Sinha, D., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

F. BIOCHEMICAL STUDIES RELATED TO BREAST CANCER

171. BIOCHEMICAL MECHANISM OF ENDOCRINE INDUCED CANCER REGRESSION

McGuire, W. L., University of Texas, Health Science Center at San Antonio, South Texas Medical School, Dept. of Physiology, *7703 Floyd Curl Dr., San Antonio, Texas,* 78284, United States of America

OBJECTIVES: Obtain tissue from human breast cancers from various clinical associates who have agreed to participate in the study and will furnish initial and follow-up records on each patient from whom tissue is obtained. Quantitatively analyze the EBP from human carcinomas representing the primary disease as well as those representing the metastatic disease. Determine the absolute concentration range of EBP per mg of cytoplasmic protein. Define biochemically the chromatin acceptor(s) for EBP in experimental animal mammary tumor systems.

172. SIALOGLYCOPROTEINS OF A METASTATIC MAMMARY TUMOR

Carraway, K. L., Carraway, C. A., Sherblom, A., Huggins, J., Oklahoma State University, School of Agriculture, Dept. of Biochemistry, *Agriculture Hall, Stillwater, Oklahoma,* 74075, United States of America

The overall goal of this program is to develop an understanding of the role(s) played by sialoglycoproteins in mammary tumors. This project will concentrate on a large, predominantly mucin-type sialoglycoprotein (ASGP-1) found in ascites forms of the 13762 mammary adenocarcinoma, but not in solid or cultured forms of the tumor. ASGP-1 molecules found in xenotransplantable (MAT-C1) and species specific (MAT-B1) forms of the tumor have been purified to homogeneity and found to have essentially identical amino acid ccmpositions, but very different carbohydrate compositions. MAT-B1 ASGP-1 can be labeled with sulfate, but MAT-C1 ASGP-1 shows little labeling. Since differences in sulfation and sialic acid content suggest differences in the control of the biosynthesis of ASGP-1 oligosaccharides, we will investigate structures of the oligosaccharides released from ASGP-1 by alkaline borohydride. We will also investigate the nature of the polypeptide chain using classical degradation techniques. The mode of association of ASGP-1 with membranes will be studied by detergent extraction and reconstitution with lipids. Antibodies against ASGP-1 and its nonglycosylated polypeptide portion will be prepared. Using cell fractionation techniques, metabolic labeling and the antibodies, we will investigate 'shedding' of ASGP-1 by the tumor cells in vitro and in vivo. Rates of component release and the nature of released components will be determined by labeling, centrifugation, electrophoresis and gel filtration. Turnover of ASGP-1 will be analyzed by pulse-chase double label experiments and compared to turnover of other cellular constituents. The presence of ASGP-1 in solid and cultured tumors will be assessed by labeling and immunological analyses. The ability of clones of the cultured tumor to produce ASGP-1 will be tested. These studies should provide considerable insight into the molecular mechanisms of expression of ASGP-1 that may be important to a role it plays in allowing the tumors to evade immune surveillance.

173. MICROANALYSIS OF CAMP ENZYMES IN HUMAN BREAST TUMORS

Rutherford, C. L., Taylor, R., Cripps, M., Gutmann, E., Woodford, T., Virginia Polytechnic Inst. & State University, School of Arts & Sciences, Dept. of Biology, *Blacksburg, Virginia,* 24061, United States of America

We plan to continue our fractionation of tumor homogenates on DEAE-Sephacel in order to separate isozymes of both alkaline phosphatase and cyclic nucleotide phosphodiesterase. For alkaline phosphatase we will determine the inhibition profiles of the separated activity peaks. In addition we will begin polyacrylamide gel electrophoretic analysis of these activity peaks using untreated fractions and fractions treated with various glycosidases e.g. neuraminidase. Further, we will use donated antiserum to determine whether any cross-reactivity exists between the isolated activity containing peaks and the isozymes of liver, placental, and intestinal origins.

Variations of the radiometric assay for phosphodiesterase suitable to the assay of microgram quantities of tissue have been evaluated. The most sensitive of these methods involved the assay of 5 microliters of sample volumes containing 1-100 micrograms of tissue extract. Tumor enzyme was found to be stable frozen at minus 76 degrees C or refrigerated overnight at a pH range of 4.8 to 9.0. Results of assays on lyophilized pieces, homogenates, and the supernatants and pellets of 27,000 x g centrifugations indicate that more than 60% of the tumor enzyme is soluble. Kinetic studies demonstrate that at least two forms of the enzyme are present in both malignant and benign samples, each with KMs in the 5 micro molar and 50 micro molar cAMP range. Preliminary fractionation data reveal two to four peaks of activity on DEAE-Sephacel, again with no significant difference appearing between the two tumor types.

BIBLIOGRAPHIC REFERENCES: Larner, E.H. and Rutherford, C.L. 1978. An artifact produced by boiling adenyl cyclase reaction mixture prior to cyclic AMP-radioimmunoassay. Anal. Biochem. 91:684-690.

174. ROLE OF DIETARY LIPIDS IN INCREASED INCIDENCE OF MAMMARY CANCER

Kidwell, W. R., Wicha, M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Epidemiological and experimental studies have implicated high dietary fat in the increased incidence of mammary cancer. Experimental systems have been established for assessing fatty acid effects on the growth of cells from normal tissue and carcinogen induced mammary tumors of rats. The studies show that all unsaturated fatty acids present in the gland in appreciable amounts promote cell growth. Both normal and tumor cells are inhibited by saturated fatty acids. Maximum growth rate of tumor cells is obtained with oleic acid while normal cells grow best in the presence of linoleic or linolenic acid. Both normal and tumor cells may be able to recruit fatty acids needed for growth from neighboring tissue. Perphenazine treatment of animals produces an increase in the relative amount of unsaturated fatty acids in the mammary gland but not in omental fat. This appears to be effected by the action of prolactin. Maps of fatty acid types at various distances from growing mammary tumors have been made. These suggest a concentration of unsaturated relative to saturated fatty acids closest to the tumors.

BIBLIOGRAPHIC REFERENCES: Kidwell, W.R., Wicha, M. and Smith, G.: Fatty acid requirements for a cell line established from a 7,12-DMBA induced rat mammary tumor. In Lippman, M. (Ed.) Conference on Hormones and Cancer. Cancer Research 38: 4091-4100, 1978. Wicha, M.S., Liotta, L.A., Kidwell, W. R.: Effects of free fatty acids on the growth of normal and neoplastic rat mammary epithelial cells. Cancer Research 39: 426-435, 1979.

175. ADVANCED BREAST CANCER - A STUDY OF SERIAL PLASMA GLYCOSLYTRANSFERASE LEVELS IN PATIENTS BEING TREATED BY CHEMOTHERAPY

Rubens, R. D., Bulbrook, R. D., Kessel, D., Imperial Cancer Research Fund, Breast Cancer Unit, *Lincoln's Inn Fields, c/o St. Bartholomew's Hospital, London WC2A 3PX, England, United Kingdom*

Levels of plasma glycosyltransferases appear to be elevated in neoplastic disease, although the relationship between enzyme levels and extent of disease has not yet been established. The level of plasma sialyl tranferase has been found elevated in patients with advanced breast cancer and in animals bearing metastasising mammary tumors. This study is directed at assessing value of measuring plasma glycosyltransferases as an index of the extent of tumor load in patients with metastatic breast cancer receiving chemotherapy. These enzymes are being estimated before starting chemotherapy and on the first day of subsequent courses of chemotherapy in patients from the previous project to see if a positive correlation with clinical response can be found.

176. BIOCHEMICAL STUDIES ON MAMMARY TUMORS (HUMAN)

Dao, T. L., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

177. SYNTHESIS OF MAMMARY SPECIFIC PROTEINS - ALPHA-LACTALBUMIN AND CASEIN IN THE GROWING AND REGRESSING MAMMARY TUMORS

Qasba, P. K., Losonczy, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The purpose of this project is to study the synthesis of alpha-lactalbumin and casein protein and their mRNAs in the mammary tumors during growth and regression. The results have shown that MTW9 mammary tumors whose growth is dependent on mammotropic stimulus, synthesize alpha-LA and caseins. Withdrawal of mammotropic hormones arrests the synthesis of alpha-LA and 42K casein proteins as early as six hours after the onset of regression. Alpha-LA mRNA levels compared with those of the growing tumors were reduced at this time of regression.

178. REGULATION OF ENZYME CONCENTRATION IN TISSUES

Knox, W. E., Kadowaki, H., New England Deaconess Hospital, Cancer Research Institute, *185 Pilgrim Rd., Boston, Massachusetts*, 02215, United States of America

Measurements of a large variety of enzymes and other cellular components will be made in each of a representative series of rat tissues, including fetal, adult and neoplastic tissues. Special attention will be given to mammary gland during the stages of lactation, especially during involution of the gland, and its arginine and protein metabolism. The objective is to classify the different compositions of tissues as the products of the differently regulated genetic programs of gene expression, and to relate these to the functional differences among the tissues. This classification will also chemically define groups of related tissues, including neoplasms. The numerical interpretations will be done by principal components and multiple discrimination analyses.

BIBLIOGRAPHIC REFERENCES: Snell, K., and Knox, W.E. Enzymes of serine metabolism in normal and neoplastic tissues. Biochem. Soc. Trans. 7, 1048-1050 (1979). Snell, K., and Knox, W.E. Synthesis of nutritionally nonessential serine in rodent fetal tissues and tumors. Ann. Clin. Lab. 1980, in press. (Association of Clinical Scientists, Spring Meeting, May 8-11, 1980, Boston, Mass.)

179. MEMBRANE PROTEINS AND LIPIDS IN MAMMARY CARCINOMA

Steiner, M. R., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

The surface membrane related characteristics which distinguish tumorigenic and nontumorigenic cells derived from mouse mammary carcinomas and normal mouse mammary glands are being determined. The tumorigenic cells exhibit alterations in several classes of fucosylated compounds, namely fucosylated amino acids, trypsin-sensitive surface fucopeptides and cell surface associated fucoproteins. As an approach to understanding the metabolism of the fucopeptides the mannose and glucose analog, 2-deoxy-D-glucose, has been used. There is a differential effect on the trypsin-sensitive surface fucopeptides when normal and transformed cells are cultured in medium supplemented with 2-deoxy-D-glucose. In addition, there are small but consistent differences in the choline phosphatides of the tumorigenic and nontumorigenic mammary cells.

180. BIOCHEMICAL ANALYSIS OF HUMAN BREAST CYST FLUID

Schwartz, M. K., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

OBJECTIVES AND APPROACH: Obtain 150 specimens of breast cyst fluid (BCF) the first year; if distinctive variations are found in biochemical, hormonal, or immunological determinations, then obtain 350 breast cyst fluids the second year and 500 the third year. Analyze these breast cyst fluids for the following components: tumor associated antigens (CEA, TPA, alpha-fetoprotein), chorionic gonadotropin, pituitary gonadotropin, minerals, protein nitrogenous constituents, DNA and polyamines, lipids, enzymes, and hormones. The hormone determinations will be performed by a subcontractor, the Institute for Steroid Research of Montefiore Hospital. Collect all initial clinical information and ongoing clinical follow-up on each patient. Follow-up women who have BCF aspirated and analyzed for at least five (5) years after aspiration to determine if they do or do not develop breast cancer. Provide biostatistical support for computer storage and statistical analysis of the above data.

181. ISOZYMES SPECIFIC TO HUMAN BREAST NEOPLASIA

Yang, N., Michigan Cancer Foundation, Dept. of Biology, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

The transformation of normal human mammary epithelium to carcinoma is currently understood primarily in morphological, growth and cell behavioral terms. Limited information is available on the biochemistry of this differentiation process. Specific gene activation and repression, reflected in expression of enzymes, their isozymes and structural proteins are the fundamental mechanisms by which the development and progression of a carcinoma are controlled. In this study, I intend to establish systematically the enzyme and isozyme differences between normal and malignant human mammary epithelium; the effect of mammotropic hormones on differential isozyme expression; and whether this is useful in diagnosis and prognosis of breast cancer.

The studies are being carried out using a defined system to culture normal and malignant mammary epithelium. Work in progress indicates that normal and malignant human mammary epithelium exhibit apparent differences in some specific enzyme and isozyme expression and such differences are applicable to normal and neoplastic breast tissues derived in vivo. Efforts are being made to identify and characterize additional isozymes unique in type or amount in neoplastic human breast cells. The possibility of using these isozymes as diagnostic and prognostic markers for breast cancer will be assessed.

182. CAMP-ACTIVATED PROTEIN KINASES IN RAT MAMMARY CANCER

Mayer, R., Joint Diseases North General Hospital, Research Institute, *1919 Madison Ave., New York, New York*, 10035, United States of America

This is part of a broader project. A summary of this subproject is not available.

183. THE INFLUENCE OF MAMMARY CARCINOMA CELLS ON THE FORMATION AND ABSORPTION OF COLLAGEN

Wirl, G., Osterreichische Akademie der Wissenschaften, Inst. fur Molekularbiologie, *Akademiestrasse 26, 5020 Salzburg, Austria*

SCOPE: The main objectives of this research are to isolate and characterize collagenase as well as any inactive forms of collagenase present in mammary tumor tissue. In the proposed project, the Contractor will: (a) attempt to extract collagenase from dimethylbenz(a)anthracene (DMBA)-induced tumors in the rat and spontaneous tumors in the C3H mouse; (b) extract the enzyme directly from tumor tissue as well as from tissue culture media; (c) purify the enzyme on Sephadex columns followed by affinity chromatography on collagen-Sepharose; (d) examine the purity of the enzyme using disc gel electrophoresis and immunodiffusion; (e) determine the collagenase activity by release of soluble radioactivity from 14C-lab . l collagen prepared from guinea pig skin; (f) determine whether the enzyme is distributed uniformly throughout the tumor or whether it is preferentially accumulated at the periphery of the tumor; (g) investigate whether collagenase is secreted as an inactive proenzyme and then activated by another enzyme; (h) determine whether collagenase is produced by epithelial cells, stromal cells or the combination of these cells. The study of human tissue may be undertaken later, depending upon the results obtained with the mouse and rat.

Another objective is to inhibit the mechanism by which cancer cells invade surrounding and distant dense connective tissue structures, including bone.

184. METABOLIC FATE OF MAMMARY CELL SURFACE GLYCOCONJUGATES

Bernacki, R. J., Porter, C. W., Russell, P., Roswell Park Memorial Inst., Dept. of Experimental Therapeutics, *666 Elm St., Buffalo, New York*, 14263, United States of America

Glycosyltransferases have been found to be elevated in the sera of cancer patients. A unique galactosyltransferase isoenzyme was discovered in the sera of colon cancer patients and differences in fucosyltransferase isoenzymes have also been found in AML patients. Therefore we have begun investigating sialyltransferase isoenzymes in the sera of mammary tumor patients. These studies should provide evidence concerning the tissue origin of the elevated sialyltransferase activity and its role in malignancy.

Together with these characterization studies of serum sialyltransferase we have attempted to synthesize specific inhibitors of sialyltransferase (Klohs, Bernacki and Korytnyk, Cancer Res. 39, 1231, 1979). Several CMP and CMP-sialic acid analogs have manifested enzyme inhibitory activity and one compound, CMP-ribodialdehyde, (Korytnyk, Angelino, Klohs and Bernacki, Eur. J. Med. Chem., in press, 1980) has shown some chemotherapeutic potential. These studies will provide more information on serum sialyltransferase in malignancy and may

lead to a sensitive diagnostic or prognostic test for mammary cancer. In addition, specific inhibitors of sialyltransferase may become useful for assessing this enzyme's role in normal and malignant cell functions.

BIBLIOGRAPHIC REFERENCES: Klohs, W.D., Bernacki, R.J., and Korytnyk, W. Effects of Nucleotides and Nucleotide Analogs on Human Serum Sialyltransferase. Cancer Research 39:1231-1238, 1979. Bernacki, R.J., and Morin, M. (1980) Membrane Metabolism as a Target in Cancer Chemotherapy. In: New Leads in Cancer Therapeutics, E. Mihich, ed., G.K. Hall and Co., Boston, in press.

185. THE EXTRACELLULAR ROLE OF THE PROTEINASE CATHEPSIN B AND OTHER PROTEINASES IN INFILTRATIVE TUMOR GROWTH AND METASTASIS

Poole, A. R., Mort, J. S., Shriners Hospital for Crippled Children, Joint Diseases Laboratory, *1529 Cedar Ave., Montreal, Quebec*, H3G 1A6, Canada

OBJECTIVE: To investigate the role of thiol proteinases in tumor invasiveness and metastasis.

APPROACH: Thiol proteinases and other proteinases are being identified, characterized and purified from tumor fluids, and from explants of human breast carcinomas and mouse mammary tumors. Antibodies to purified thiol proteinases are being used to identify these enzymes. The ability of thiol proteinase to alter cell surfaces and to degrade connective tissue macromolecules, including basement membrane components, is being studied.

PROGRESS: A thiol proteinase is selectively and excessively secreted from human mammary carcinomas and mouse tumors compared with non-malignant breast tissue and human fibroadenomas of the breast. This proteinase resembles cathepsin B but is different in that it is stable at neutral pH and does not react with a monospecific antiserum to human liver cathepsin B.

186. REGULATION OF FATTY ACID BIOSYNTHESIS IN MAMMARY TUMORS

Ahmad, F., Altman, N. H., Papanicolaou Cancer Research Inst., *1155 14th St. N.W., Box 23-6188, Miami, Florida*, 33136, United States of America

These investigations are aimed at a better understanding of the regulation of fatty acid biosynthesis in normal rat mammary gland and mammary tumors. To achieve this objective we are conducting the following studies: a) Whereas the activity of hepatic acetyl CoA carboxylase has been shown to be regulated by phosphorylation/dephosphorylation of the enzyme molecules, it is not yet certain whether the activity of rat mammary gland acetyl CoA carboxylase is also controlled by a similar mechanism. Therefore studies will be conducted on the regulation (short-term) of rat mammary gland carboxylase. b) The regulation of acetyl CoA carboxylase and fatty acid synthetase in explants of normal mammary gland and mammary tumors will be investigated. c) Using certain acyl CoA analogs, we will explore the mechanism of reaction catalyzed by rat mammary gland fatty acid synthetase.

BIBLIOGRAPHIC REFERENCES: Clements, P.R., Barden, R.E., Ahmad, P., and Ahmad, F. Affinity Labeling of Fatty Acid Synthetase from Lactating Rat Mammary Gland with S-(4-Bromo-2,3-Dioxobutyl)-CoA: Evidence for a 'Half-of-the-Sites' Catalytic Mechanism, Biochem. Biophys. Res. Commun. 86, 278-284 (1979). Ahmad, P., Russell, T.R., and Ahmad, F. Increase in Fatty Acid Synthetase Content of 3t3-L Cells Undergoing Spontaneous and Chemically Induced Differentiation to Adipocytes, Biochem. J., 182, 509-514 (1979).

187. CHARACTERIZATION OF HUMAN BREAST CANCER GLYCOPROTEINS

Bolognesi, D. P., Langlois, A. J., Collins, J. J., Roloson, G. J., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina*, 27710, United States of America

We have developed a unique model system for passive serum immunotherapy where immunological management of a highly lethal tumor, the ascites form of murine adenocarcinoma 755a, can be carried out reproducibly and with remarkable efficiency. Information concerning the mechanism of AD755a immunotherapy will be obtained through the isolation and characterization of the AD755a tumor-associated antigens and the serum factors responsible for providing protection. The type-C oncornavirus associated with the AD755a tumor will be isolated and the role of its structural polypeptides in the process will be determined.

The identity of AD755a tumor surface antigen(s) which induce and/or react with the protective serum will be analyzed by several analytical immunoassays, and attempts will be made to produce a protective serum using purified AD membrane antigens. The nature of the protective serum factor will be determined by isolating the class and subclass of antibody responsible for protection and the possible role of anti-idiotype antibodies in the serum will be analyzed.

BIBLIOGRAPHIC REFERENCES: Haagensen, D.E., Jr., Roloson, G., Collins, J.J., Wells, S.A. Jr., Bolognesi, D.P. and Hansen, H.J. (1978). Immunological control of the ascites forms of murine adenocarcinoma 755. I. Protection with syngeneic immune serum or lymphoid cells. J. Nat. Cancer Inst. 60:131-139. Collins, J.J., Roloson, G., Haagensen, D.E. Jr., Fischinger, P.J., Wells, S.A. Jr., Holder, W.D. Jr., and Bolognesi, D.P. (1978). Immunologic control of the ascites form of murine adenocarcinoma 755.

188. CONTROL OF MILK PROTEIN SYNTHESIS IN MAMMARY TUMORS

Marzluff, W. F., State University System of Florida, Florida State University, School of Arts & Sciences, Dept. of Chemistry, *205 Wildwood Dr., Tallahassee, Florida*, 32306, United States of America

Continuous cell lines have been established from several mammary tumors. Each line has its own characteristic morphology, ranging from a cuboidal to a fibroblast-like cell. The tumors include both carcinogen-induced (DMBA) and spontaneous tumors. The milk proteins, casein and alpha-lactalbumin, have been purified and antibodies prepared. The mRNAs for these proteins have been purified and cDNAs prepared.

Using a sensitive radioimmunoassay we have detected alpha-lactalbumin in the tumors as a small amount of the cell protein. The tumors also synthesize alpha-lactalbumin and casein when incubated in 35S-methionine in organ culture. The level of these proteins is greatly reduced in ovariectomized rats although the tumors grow well. The levels of milk proteins are reduced in the cultured cells, although the cells are still positive for casein and alpha-lactalbumin by immunofluorescence.

Using isolated nuclei active in transcription we are studying the production of the mRNAs in both lactating mammary gland and tumor cell nuclei.

189. GALACTOSYLTRANSFERASE AND LACTALBUMIN OF THE LACTOSE SYNTHETASE SYSTEM

Ebner, K. E., University of Kansas, Medical Center, School of Medicine, Dept. of Biochemistry, *39th St. & Rainbow Blvd., Kansas City, Kansas*, 66103, United States of America

The major thrust of this work is to learn the structural and mechanistic properties of a galactosyltransferase which, in conjunction with alpha-lactalbumin, is involved in the biosynthesis of lactose. Alpha-lactalbumin isolated from the milk of most species does not contain carbohydrate. In contrast, alpha-lactalbumin isolated from rat milk is fully glycosylated and the question is why this is the case. Also, there is considerable interest in the mechanism of glycosylation of proteins and in particular the involvement of lipid intermediates in the transfer of carbohydrates to an asparagine residue. Rat alpha-lactalbumin is an excellent model system for such studies since it is easy to isolate and can be synthesized in slices, tissue pieces and certain mammary tumors. In addition, its synthesis is under hormonal control. For such studies to be meaningful it is necessary that the amino acid sequence of rat alpha-lactalbumin and the sequence of the carbohydrate units be completed. Progress in both of these areas has been made. The amino acid and carbohydrate sequence of rat alpha-lactalbumin will be completed. Studies on galactosyltransferase with respect to the carbohydrate units and chemistry of the active site will be continued.

190. STUDIES OF MAMMARY TUMOR PEROXIDASE

DeSombre, E. R., Lyttle, C. R., University of Chicago, School of Medicine, Dept. of Cancer Research, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

The overall objective of this investigation is to gain a better understanding of the induction, regulation and physiologic role of peroxidase activity in the normal and neoplastic mammary gland; further, to evaluate the increased peroxidase activi-

ty and iodine uptake of mammary tumors for their diagnostic potential and correlation with hormone response of the tumors. To this end the enzymatic peroxidase activity and protein iodination will be studied: 1) in the rat mammary gland under various hormone influences, during iodine deficiency, and during mammary carcinogenesis and 2) in rat mammary tumors. Peroxidase activity will be assayed in salt extracts of the tissues using the guaiacol oxidation assay and the activity compared with in vivo and in vitro tissue uptake of radiolabelled iodine and iodine incorporation into protein. Qualitative changes in iodinated proteins will be assessed by two-dimensional gel electrophoresis. The effects of hormones, especially estrogen, progesterone and prolactin, on the induction and regulation of peroxidase activity in normal and neoplastic mammary tissues will be elucidated. The effect of various endocrine manipulations of mammary tumor growth will be correlated with tumor peroxidase activity. Peroxidase enzymes from mammary gland and tumors will be purified and characterized and variations in peroxidase isoenzyme patterns in these tissues will be studied. Kinetics of peroxidase synthesis, assembly from apoenzyme and porphyrin, and degradation will be investigated.

BIBLIOGRAPHIC REFERENCES: C.R. Lyttle, S.M. Thorpe, E.R. DeSombre and J.L. Daehnfeldt: Peroxidase activity and iodide uptake in mouse mammary tumors. J. Nat. Cancer Inst., 1979, in press. C.R. Lyttle, R.V. Garay and E.R. DeSombre: Ontogeny of estrogen inducibility of uterine peroxidase, J. Steroid Biochem., 1979, in press.

191. SERUM LIPOPROTEINS IN PATIENTS WITH BREAST CANCER

Aladjem, F., Lee, Y., University of Southern California, School of Medicine, Dept. of Microbiology, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

Significant serum lipoprotein concentration differences have been reported to occur between patients with mammary carcinoma, normal individuals with a family history of cancer, and normal individuals without family history of cancer.

The objective of this work is to study the chemistry of the serum lipoproteins of individuals in these three groups. The first objective will be to ascertain whether the differences are quantitative only or whether chemically different aproproteins, and consequently lipoproteins, occur. If qualitative differences are found, then the next objective will be to determine the chemical basis of the differences.

192. NUTRIENT UPTAKE IN NORMAL AND TUMOROUS MAMMARY GLAND

Neville, M. C., University of Colorado, Medical Center, School of Medicine, Dept. of Physiology, *4200 E. 9th Ave., Denver, Colorado*, 80262, United States of America

Investigation into the specificity of amino acid transport in mouse mammary gland will be continued. A quantitative test for the specificity of the A and L sites will be developed and applied to glands from pregnant and lactating mice, as well as to mouse mammary tumors in vitro.

BIBLIOGRAPHIC REFERENCES: Lobitz, C.J., and Neville, M.C. (1977) Control of amino acid transport in the mammary gland of the pregnant mouse. J. Supramolec. Struct. 6: 355-362.

193. ACID MUCOPOLYSACCHARIDES IN HUMAN BREAST TISSUE

Clement, I. P., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

194. BIOCHEMICAL CHANGES IN THE DEVELOPMENT OF BREAST CANCER

Kiricuta, I., Bojan, O., Comes, R., Institutul Oncologic Cluj-Napoca, Dept. of Biochemistry, *34-36 Republicii, 3400 Cluj-Napoca, Romania*

The levels of haptoglobin, sialic acid and fucose are investigated in the sera of patients with malignant and benign tumors of the breast and in those of healthy subjects. The determination of these biochemical parameters is carried out according to standard methods. Fucose is determined as described by Dische and Shetler, sialic acid according to Seiter and Gerstenfeld, and haptoglobin by using the peroxidase activity as indicated by Owen.

Preliminary results indicate that the levels of haptoglobin, sialic acid and fucose increase according to the development of malignancy. The increase of these parameters as determined at different time intervals within a period of 1.5 years after the surgical removal of the primary tumor preceded by about two months the radiological diagnosis of metastases. Therefore, these simple biochemical determinations appear to permit early detection of metastases and the bilateral extension of cancer of the breast.

G. OTHER CELL BIOLOGY INCLUDING CELL CULTURE AND MARKER STUDIES

195. THE FIBROTIC RESPONSE TO HUMAN BREAST CARCINOMA

Stern, R., Tseng, S., University of California, San Francisco Campus, School of Medicine, Dept. of Pathology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Many human tumors are associated with a fibrotic response, termed the desmoplastic reaction. The most profound reaction is elicited by human breast tumors, particularly the infiltrating ductal carcinomas. It is not known whether this connective tissue reaction is the product of fibroblasts in the invaded host tissue, is synthesized by the malignant epithelial cells of the tumor, or is perhaps dependent on an interaction between the two cell types. This proposal attempts to answer these questions.

Established lines of human breast tumor cells obtained originally from malignant effusions from women with disseminated disease will be examined for their ability to elaborate collagen into tissue culture medium. Normal human skin fibroblasts will also be examined for their ability to synthesize collagen. Combinations of these cells in mixed culture and the effects of conditioned media in various combinations will be examined.

In preliminary studies, a polypeptide factor which underlies this desmoplastic reaction has been identified. The immediate goal of the current studies will be to isolate and purify this peptide, and to prepare an antibody which will permit development of a radioimmunoassay. This may provide a clinical marker for identifying patients with occult breast carcinomas.

196. IN VITRO STUDIES ON MAMMARY NEOPLASTIC PROGRESSION

Asch, B. B., Beth Israel Hospital, Dept. of Pathology, *330 Brookline Ave., Boston, Massachusetts*, 02215, United States of America

Normal, dysplastic, and malignant mammary tissues are all composed of heterogeneous populations of cells. The objectives of the proposed research are to delineate specific cellular markers for distinguishing the different types of epithelial cells in these tissues and to identify and characterize cell surface factors which might be involved in mediating the interactions of these cells with each other and with extracellular substrata to which they attach. The workscope of this proposal includes three basic approaches utilizing in vitro methodology. First, lectins in conjunction with other agents will be used to attempt to define common and unique surface-related features of the different types of mammary cells. Second, factors involved in homotypic and heterotypic intercellular adhesion and in cell-substratum adhesion will be investigated to determine if the normal, dysplastic, and malignant cells differ in these functions. Third, extracellular material apparently produced by the cells will be studied to establish if the material is part of an extracellular matrix formed by the cells and if there are qualitative or quantitative differences in its production by the different cell types. Results of these endeavors hopefully will lead to a more comprehensive understanding of the roles and fate of each epithelial cell type during the pathogenesis of mammary neoplasia.

BIBLIOGRAPHIC REFERENCES: Comparative Responses of Normal and Malignant Mouse Mammary Cells to Modulation of Surface Properties, by B.B. Asch, D. Medina, F. Kretzer, J.L. Connolly, and B.R. Brinkley, Cancer Research (July or August 1980). Cell Markers for Mouse Mammary Preneoplasias by D. Medina and B.B. Asch, in Advances in Breast Cancer Research, M. Brennan (ed.), Academic Press, N.Y. (1980 or 1981), in press.

197. CHARACTERIZATION OF HUMAN MAMMARY CELLS

Stampfer, M. R., University of California, Berkeley Campus, Lawrence Berkeley Lab., Biology & Medicine Division, *Berkeley, California*, 94720, United States of America

We are developing an in vitro human mammary cell culture system to be used for studies of human cellular differentiation and carcinogenesis. In particular, we will investigate: (1) the effects of potential growth promoters on human mammary epithelial cells; (2) the effects of hormones and cell substrates on expression of mammary-specific products (medium chain fatty acids, alpha-lactalbumin, casein); (3) properties that may differentiate between normal and malignant mammary epithelial cells (surface properties, tumor-specific antigens); and (4) metabolism of benzo-a-pyrene by these cells.

198. PEROXIDASE AS A MARKER ENZYME IN ESTROGEN-DEPENDENT BREAST CANCER

Jellinck, P. H., Queen's University, Dept. of Biochemistry, *King St. W., Kingston, Ontario*, K7L 3N6, Canada

OBJECTIVE: To obtain evidence for the proposal that peroxidase is a specific marker enzyme in breast tumors which display growth-dependency on estrogens.

APPROACH: Mammary tumors will be induced in female Sprague-Dawley rats by gastric instillation of 7,12-dimethylbenz(a)anthracene (DMBA) and the response of the tumors ascertained following ovariectomy or treatment with anti-estrogens (Nafoxidine, Tamoxifen or MER-25). Samples of the tissue will then be assayed for peroxidase, NADH oxidase and estrogen metabolizing activities and the molar concentrations of high affinity receptor sites for estradiol and also progesterone determined. The correlation between these properties of the tumor will be investigated and attempts made to purify and characterize the tumor peroxidase.

PROGRESS: The peroxidase activity and concentration of high affinity estrogen receptor sites was determined in DMBA-induced rat mammary tumors. Only a moderate correlation was obtained between the activity of this enzyme and nuclear estrogen receptor concentration and no better correlation was shown between peroxidase activity and the levels of cytosol estrogen receptor before and after treatment with estradiol. These results are compatible with recent concepts of estrogen action and do not exclude the possibility that peroxidase provides a better indication of hormone responsiveness than the concentration of estrogen receptor. As proposed for the induced protein (Notides and Gorski) or the progesterone receptor (Horwitz and McGuire), the biosynthesis of an end-product like peroxidase which is dependent on estrogen shows that an intact transcriptional system is present and such tumor cells are therefore likely to respond to endocrine therapy (Lyttle, Thorpe, DeSombre and Daehnfeldt, J. Natl. Cancer Inst. 62, 1031, 1979; Jellinck, Newcombe and Keeping, Adv. Enzyme Regul. 17, 325, 1979).

199. IMMUNODIAGNOSTIC MARKERS FOR BREAST CARCINOMA

Chawla, R. K., Emory University, School of Medicine, Dept. of Medicine, *1364 Clifton Rd. N.E., Atlanta, Georgia*, 30322, United States of America

OBJECTIVES: Prepare a supply of pure EDC1, pure IATI (inter-alpha trypsin inhibitor), and high titer antiserum to EDC1; the EDC1 method shall be standardized. Utilize CIEP to measure the plasma and urine levels of EDCU and LATI in normal women, those with benign diseases, and those with breast carcinoma. Perform plasma/urine EDC1 measurement preoperatively on patients with a localized breast mass and follow those patients postoperatively in consultation with and approved by the project officer.

200. MICROCIRCULATION AND MOLECULAR TRANSPORT IN MAMMARY CANCER

Gross, J. F., University of Arizona, School of Mines, Dept. of Chemical Engineering, *1501 N. Campbell Ave., Tucson, Arizona*, 85721, United States of America

The objective of the investigations proposed is to quantitatively characterize, by means of direct measurements, the transport properties of solid tumor microcirculation in vivo and in situ. The work proposed includes the implantation of a biological tumor sandwich window preparation which can be studied by existing and available microcurculatory methods, using the BL-DMBA No. 1 mammary adenocarcinoma tumor in F344 rats which is well-characterized in terms of histology, growth rate and radiation sensitivity. The expertise developed in this area will be subsequently utilized to develop a nude mouse mammary carcinoma sandwich preparation, which will be studied by the same methods.

201. DIETARY FAT AND BREAST CANCER (RATS, TISSUE CULTURE)

Wynder, E. L., American Health Foundation Inc., *320 E. 43rd St., New York, New York*, 10017, United States of America

This is part of a broader project. A summary of this subproject is not available.

202. BLOOD GROUP SUBSTANCES IN MALIGNANT AND BENIGN BREAST CYST FLUIDS

McGinniss, M. H., DeMoss, E. V., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, Clinical Center, Surgical Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Breast cyst fluids are being analyzed for the presence of soluble blood group substances, both serologically and biochemically. These data will be correlated, at a later date, with breast cyst fluid analysis for malignancy. If a consistent difference is noted between malignant and non-malignant cyst fluids, it may serve as a marker for patients at risk.

203. GROWTH/PASSAGE OF MAMMARY EPITHELIAL CELLS IN CULTURE

Sato, G. H., University of California, San Diego Campus, School of Medicine, Dept. of Biology, *P.O. Box 109, La Jolla, California*, 92093, United States of America

OBJECTIVE: Devise a medium which will support the growth of normal mammary epithelial cells by proceeding in the stepwise manner of first finding the exact requirements of established tumor lines, then of primary tumor cultures, and finally - of normal epithelia. The idea is that established tumor lines have lost some of the requirements of normal cells. In addition, we think that anything required by established tumor cells will be required by primary tumor cells and normal epithelia. We also think that by proceeding with defined, serumless media, we will eliminate inhibitory factors which have hampered attempts to culture normal epithelia. Finally, when serum-free media are used for primary tumor cells and normal tissue, the fibroblasts die after two to three days so that only the growth requirements of epithelial cells are studied.

204. GROWTH/PASSAGE OF MAMMARY EPITHELIAL CELLS IN CULTURE

Misfeldt, D. S., Stanford University, School of Medicine, Dept. of Medicine, *Stanford, California*, 94305, United States of America

OBJECTIVE: Define the primary problems in the establishment of normal mammary epithelial cell lines. Humans and mice are the species to be used. This presentation will focus on the rationale and comparable related work limited to those species. There are but a few cell lines established from normal mammary epithelia. In the mouse, Owens et al. (1974) isolated a mammary epithelial cell line from an outbred strain. Subsequent in vitro transformation cannot be determined by implantation into isologous hosts since this strain is not highly inbred. The cells have retained in vitro many of the in vivo features of transporting epithelia such as apical/basal orientation, occluding tight junctions, and transepithelial transport.

205. BIOLOGIC AND VIROLOGIC CHARACTERIZATION OF BREAST CANCER CELL LINES

Young, N. A., Arya, S. K., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Oncology & Molecular Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A detailed investigation of retroviruses in four new human breast cancer cell lines did not yield unequivocal evidence for viral functions. The cell lines did not display particle-associated RNA and RNA directed DNA polymerase. The genome of these cells did not contain mouse mammary tumor virus (type B) related DNA sequences. Similarly, electron microscopic search for budding particles gave negative results.

In a related study with mouse model system, we found that mouse breast tumor cells (Mm5mt/c1) display age-related switches in retroviral gene expression. While the early passage cells produce mainly type B virus (MMTV), the late passage cells produce predominantly ecotropic type C virus. Though type B virus production is shut down, the late passage cell continues to synthesize type B viral RNA. The lesion appears to lie at the level of processing of type B viral mRNA.

BIBLIOGRAPHIC REFERENCES: Job, L., Carter, W.A., and Arya, S.K.: Reverse transcriptase activity in extracts of human prostatic tissues. Oncology 35: 202-205, 1978.

206. CHARACTERIZATION OF HUMAN MAMMARY TUMOR CELLS

Stampfer, M. R., Peralta Hospital Assn., Cell Biology Section, *450 30th St., Oakland, California,* 94609, United States of America

The objective of the proposed research is to develop conditions for in vitro culture of human mammary cells that would facilitate the use of this human cell system in investigations concerning differentiation and carcinogenesis. We have already established conditions that permit short-term growth of human mammary epithelial and myoepithelial cell cultures from many different types of tissues (reduction mammoplasties, benign tumors, mastectomies). We now propose to examine these cells for (1) growth response to different hormones, hoping to determine optimal concentrations of required nutrient factors and thus promote increased cell growth in vitro; (2) expression of mammary specific properties, such as alpha-lactalbumin, casein, and lactose synthesis, in response to different hormonal environments and different culture support systems; (3) properties which may be associated with carcinogenic transformation. These studies may allow us to provide well-characterized cell populations for numerous areas of research.

207. BIOLOGIC MARKERS IN BREAST CANCER PATIENTS

Schwartz, M. K., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York,* 10021, United States of America

Determine the concentrations of a selected group of constituents in breast tissue obtained at mastectomy and to assay for these same substances in the serum of the patients obtained before their surgery and then sequentially on a scheduled basis over a period of time. Our aim is to find a constituent in cancerous breast tissue that is present in a concentration different from that in normal breast tissue and then to try to establish if changes in these concentrations in the breast tissue are reflected in the serum and whether the course of the disease is reflected in the serum pattern. We are now routinely obtaining in our laboratory breast tissue for analysis of estrogen receptor protein. It is our aim to determine in the cytosol and pellet of these tissues a variety of substances whose assays are in use in our laboratory. In addition, we can make available specimens for analysis in other laboratories

208. WORKSHOP ON NEW STRATEGIES IN BREAST CANCER RESEARCH - APPLICATION OF BREAST CELL CULTURE SYSTEMS

Rich, M. A., Michigan Cancer Foundation, *110 E. Warren St., Detroit, Michigan,* 48201, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

209. CELL-CELL INTERACTIONS - HUMAN MAMMARY CELLS IN CULTURE

Hackett, A. J., Peralta Hospital, Peralta Cancer Research Inst., *450 30th St., Oakland, California,* 94609, United States of America

The effects of cocultivation of human mammary stromal and glandular cells will be studied. Epithelial, myoepithelial, and fibroblastic cells derived from normal, atypical, and carcinomatous tissues will be examined for expression of specific markers possibly correlated with malignant and metastatic potential. Specifically, cells grown alone and in mixed cultures will be evaluated for: 1) Cellular and colony morphology by light microscopy and scanning electron microscopy; 2) metabolic transfer between cells and; 3) surface proteins (LETS, large external transformation sensitive). These studies may elucidate the role of cell-to-cell interactions in the invasion of normal tissue by malignant cells and in the normal differentiation of epithelial cells for secretory activity.

210. DETECTION OF HUMAN MILK PROTEIN RNA TRANSCRIPTS IN MAMMARY TUMOR BIOPSIES

Craig, R. K., Davis, M. S., Courtauld Inst. of Biochemistry, *London, England, United Kingdom*

OBJECTIVE: Development of a nucleic acid hybridization assay for the routine clinical detection of human milk protein RNA transcripts in mammary tumor biopsies.

APPROACH: Isolate and clone, using standard DNA recombinant techniques, mRNA sequences coding for human milk protein. These will then be used to identify the presence or absence of RNA molecules containing milk protein sequences in mammary tumor biopsies.

211. OSTEOTROPISM OF MAMMARY CARCINOMA METASTASIS

Mundy, G. R., Eilon, G., Raisz, L. G., University of Connecticut, Health Center, School of Medicine, Dept. of Medicine, *Farmington Ave., Farmington, Connecticut,* 06032, United States of America

In vitro models have been developed to study the cellular and molecular mechanisms of formation of osseous metastases in patients with breast cancer. We have found that human breast cancer cells resorb bone directly. We now plan to examine the molecular mechanism of breast cancer cell-mediated bone resorption, including the characterization of the factors released by cultured human breast cancer which resorb bone, determination of the relationship between mineral release and matrix resorption, and the roles of lysosomal enzymes and collagenase. We also plan to determine the mechanism of bone resorption in two rat breast tumor models associated with hypercalcemia. We plan to continue our studies on the bone-derived chemotactic factor for breast-cancer cells, including its biological and chemical characterization and identification of agents which inhibit it. We will also continue our studies on the role of prostaglandins in the hypercalcemia of malignancy and examine supernatants of cultured human breast cancer cell lines for factors which stimulate new bone formation.

212. COLONIZATION POTENTIAL OF MAMMARY TUMOR CELLS

Tarin, D., John Radcliffe Hospital, Dept. of Pathology, *Headington, Oxford, England, United Kingdom*

OBJECTIVE: To examine the surface properties and behavioral characteristics of mammary tumor cells and relate them to whether the cells produce distant secondary deposits on I.V. inoculation.

APPROACH: Naturally occurring murine mammary tumors are disaggregated into cell suspensions by mincing and digestion with collagenase. After washing and counting the cells an aliquot is removed for histological and electron microscopical examination and cell culture. The remainder are injected I.V. into the original tumor bearer and other recipients. The mice are autopsied 3-6 weeks later. The degree of correlation of various properties, e.g., types of cells in inoculum, mitotic rate, agglutinability, condition of glycocalyx and of cell surface antigens, with colonization behavior in vivo will be studied. Cells from metastatic deposits will be compared with those from parent primary tumors and from non-neoplastic mammary glands.

213. CULTURE OF PRENEOPLASTIC MAMMARY CELLS OF THE MOUSE

Hosick, H. L., Anderson, L. W., Washington State University, School of Arts & Sciences, Dept. of Zoology, *Administration Bldg., Room 101, Pullman, Washington,* 99163, United States of America

The project consists of analyses of growth and differentiation in a preneoplastic lesion of the mouse mammary gland, the hyperplastic alveolar nodule (HAN). Cell culture methodology will be used to compare selected properties of these HANs with the same properties of cells from normal (midpregnant) and neoplastic epithelium of the mouse mammary gland. Emphasis will be on regulation of mammary function, including the progression to neoplasia, by exogenous factors, hormones and mesenchymal stroma. Accordingly, the three types of cell will be

grown in media containing, in various permutations, serum, mesenchymal extract, and several hormones known to govern mammary function. Growth responses will be carefully compared under these several conditions, by measuring DNA accumulation. The regulation of lactose production by the same culture medium factors will be determined. These studies should pinpoint key changes in exogenous regulation of growth and development during neoplastic progression. The idea is to learn just how differentiated function ceases as neoplastic growth commences, since the two seem to be mutually exclusive in mouse mammary tumor cells.

214. CALCITONIN AS A MARKER FOR CANCER OF THE BREAST

Silva, O. L., Becker, K. L., Chisholm, R., Howard University, School of Medicine, Dept. of Medicine, *2400 6th St. N.W., Washington, District of Columbia*, 20001, United States of America

Using a sensitive and specific radioimmunoassay we have developed for the determination of human calcitonin (HCT) in unconcentrated peripheral blood, we propose to study HCT levels in patients with cancer of the breast. Calcitonin is a hypocalcemic, hypophosphatemic polypeptide hormone secreted by C cells of mammalian thyroid. In 1973, we reported finding increased levels in 35% of cancer patients with spontaneous hypercalcemia. During a study of normocalcemic patients with bronchogenic cancer, it became apparent that over 69% of these patients had calcitonin levels above normal. Was this hypercalcitonemia a response to bone resorption and impending hypercalcemia? Could calcitonin be used as a marker in cancer? Coombes et al. confirmed the high incidence of hypercalcitonemia in bronchogenic cancer and, in addition, showed that 100% of the breast cancer patients with skeletal metastases, studied by them, had high values of blood calcitonin which could be augmented by calcium infusion. In our laboratory a preliminary study of 13 women with metastatic breast cancer in the clinic at the Howard University Hospital showed that 69% had elevated HCT levels. (Many of these patients had undergone prior therapy). We intend to correlate HCT levels with the duration of disease, disease-free period, presence and extent of bone metastases, presence of extra-skeletal metastases, effect of therapy and prognosis. Perhaps HCT may prove to be a marker for breast cancer.

BIBLIOGRAPHIC REFERENCES: Silva, O.L., Baylin, S., Wells, S., Snider, R.H., Moore, C.F. and Becker, K.L.: Urinary calcitonin as a test for medullary thyroid cancer: a new screening procedure, Ann. Surg. 189(3): 269-274, 1979. Becker, K.L., Bivins, L.E., Radfar, R.H., Snider, R.H., Moore, C.F. and Silva, O.L.: Study of calcitonin heterogeneity using a radioreceptor assay. Horm. Metab. Res. 10(5): 457-458, 1978.

215. BIOLOGIC MARKERS IN BREAST CANCER PATIENTS

Sussman, H. H., Stanford University, School of Medicine, Dept. of Pathology, *Stanford, California*, 94305, United States of America

OBJECTIVES: Conduct a longitudinal study of biologic markers in breast cancer patients. Evaluate tissue and serum markers currently proposed as being potentially useful and develop a new set of markers from placental membrane proteins and glycoproteins and investigate their presence in serum and tissue. These studies will be coordinated with a well defined clinical study of breast cancer sponsored by the Northern California Cancer Program and conducted by the Northern California Oncology Group. This will provide a set of patients whose clinical care will be rigorously controlled and will also ensure direct control of specimens and accurate patient information. There will be twelve major institutions participating in these studies. The NCOG protocols will evaluate the patients by routine tests, and these will be complemented by our evaluation of the markers that will be measured in this protocol.

216. STUDIES OF NORMAL AND MALIGNANT HUMAN BREAST EPITHELIUM IN VITRO

Blackstein, M. E., University of Toronto, Faculty of Medicine, Dept. of Anatomy (Histology), *92 College St., Toronto, Ontario*, M5S 1A1, Canada

The summary for this project is under entry no. 122.

III. IMMUNOLOGY OF BREAST CANCER

A. ANTIGENS, ANTIBODIES AND IMMUNE COMPLEX STUDIES

217. MILK-DERIVED ANTIGENS IN BREAST CANCER

Goldsmith, I. J., Young, S., Gilbert, J. D., Imperial Cancer Research Fund, Pathology Laboratory, *Lincoln's Inn Fields, c/o St. Bartholomew's Hospital, London WC2A 3PX, England, United Kingdom*

Work has continued on the separation and purification of milk-derived antigens that can be detacted in histological sections of breast cancer.

In particular, attempts have been made to isolate one highly antigenic protein, antibodies to which have been found as contaminants in antisera prepared both by us and by other workers (ICRF Scientific Report 1978 - report 248).

Fresh human milk samples were processed to give the following fractions: a casein fraction; a proteose peptone fraction; a milk fat globule membrane fraction; and a whey protein fraction. Initial tests suggested that the proteose peptone fraction would be an ideal starting material but difficulties were experienced, probably due to denaturation during the preliminary separation.

A fraction containing the contaminant protein was prepared by anionic ion exchange chromatography and gel filtration chromatography. SDS polyacrylamide gel analysis showed it to be similar to the 'bound' protein eluted from an affinity chromatography column prepared with antibodies to the contaminant protein.

Experiments are also in hand to isolate the contaminant protein from the whey protein fraction by affinity chromatography, using a combination of immobilized lectins, and to investigate the possibility of the production of monoclonal antiboides to the contaminant protein.

218. DETECTION OF ANTIGEN-ANTIBODY COMPLEXES IN BREAST CANCER

Medof, M. E., University of Chicago, School of Medicine, Dept. of Medicine, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

OBJECTIVES: Assay a statistically adequate number of sera from patients with breast cancer, benign breast disease, and normal volunteers for IC's by employing multiple techniques for IC detection and quantitation which have been developed in and are currently in operation in our laboratory. Our multiple techniques will permit differentiation between soluble and insoluble IC's and between complement bearing and IgG-Fc accessible IC's. To correlate presence or absence, or levels where applicable, of individual IC subpopulations with tumor progression, response to therapeutic intervention, and clinical course, otherwise. Data will be analyzed according to clinical stage of the patient, histologic type of the tumor, histology of regional lymph nodes, ovarian and other hormonal status of the patient, and the presence or absence of estrogen receptors on tumor cells.

219. MURINE MAMMARY TUMOR-SPECIFIC ANTIGENS (MICE, RABBITS)

Hollis, V. W., Howard University, Graduate School of Arts & Sciences, Dept. of Zoology, *2400 6th St. N.W., Washington, District of Columbia*, 20001, United States of America

This is part of a broader project. A summary of this subproject is not available.

220. CELL MEDIATED IMMUNITY TO HUMAN BREAST TUMOR ANTIGENS

Kadish, A. S., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Pathology, *1300 Morris Park Ave., Bronx, New York*, 10461, United States of America

This is part of a broader project. A summary of this subproject is not available.

221. STUDIES ON SOLUBLE ANTIGENS FROM MTV INDUCED MAMMARY TUMOR

Laing, C. A., Howard University, School of Medicine, Dept. of Oncology, *2400 6th St. N.W., Washington, District of Columbia,* 20001, United States of America

The purpose of this study is (a) to isolate tumor-specific and virus-related antigens from a crude perchloric acid extract of MTV-induced mammary tumors of BALB/cfC3H mice, (b) to assess the biological activities of the isolated components, (c) to determine the cell types involved in the DNA synthetic response of tumor bearing mouse spleen cells to components of the extract, (d) to determine the changes of T cell, B cell and macrophage population with growth of syngeneic transplants of the tumor, (e) to determine the effect of tumor growth on DNA synthetic activity, (f) to determine the nature of serum factors inhibiting the DNA synthetic response and to isolate the serum inhibitory factors, (g) to determine the mechanism of action of the serum inhibitory factors, and (h) to determine the effect of surgical removal of the tumor on lymphocyte response to the tumor extracts, on the changes in T cell, B cell and macrophage populations, and on serum inhibition of DNA synthesis.

222. PURIFICATION OF BREAST TUMOR ASSOCIATED ANTIGENS

Oldham, R. K., Vanderbilt University, School of Medicine, Dept. of Medicine, *21st Ave. S. & Garland, Nashville, Tennessee,* 37232, United States of America

OBJECTIVE: Isolate and purify the tumor associated antigen of the MCG-7 breast cancer derived tissue culture line for use in generating heteroantisera, to lead to the development of a radioimmunoassay for that antigen for use in immunological testing of patients with premalignant breast disease as well as patients with clinical carcinoma of the breast. Plans for monitoring of the antigen during purification and for analysis of clinical data shall be provided to the project officer for approval before such work is under way.

223. DETECTION OF EARLY ANTIBODIES TO MAMMARY CARCINOMA

Blair, P. B., University of California, Berkeley Campus, School of Letters & Science, Dept. of Bacteriology & Immunology, *Berkeley, California,* 94720, United States of America

The early antibody response to tumor-associated antigens is being studied in an experimental animal tumor system. Mammary tumor incidence in females neonatally infected with the mammary tumor virus (MTV) is very high in some strains of mice and low in others. Thus, both retrospective studies for the characterization of antibody responses and prospective studies for testing antibody patterns predictive of eventual tumor development are feasible.

Serum samples from females of a strain with high tumor incidence (neonatally infected with MTV) are collected and frozen at regular intervals over the life span of the animals. As each female develops a mammary tumor which can be used to provide target tumor cells for in vitro testing, the serum samples are tested for the presence of antibodies directed against the various tumor-specific and virus-associated tumor antigens. A number of detection procedures are used, such as tests for blocking factors, cytotoxic antibodies, and virus-precipitating antibodies.

Since females bearing autochthonous rather than transplanted tumors are being used, the immune response in the natural course of a disease is being studied. These tumors are induced by a virus, and we suspect that part of the antibody response may result from a host-virus interaction which is not predictive of eventual tumor development; use of the model system will permit a qualitative as well as quantitative analysis of a complex response which may be directly applicable to human tumor systems.

224. ANTIGEN-ANTIBODY COMPLEXES IN BREAST CANCER

Chu, T. M., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

The objective of this study is to assay soluble antigen-antibody complexes in serum of patients with breast cancer using C1q binding radioimmunoassay, Raji cell radioimmunoassay, and (rabbit) rheumatoid factor assay. The serum specimens will be obtained from breast cancer patients, fibrocystic breast disease patients and breast cancer families as well as from appropriate healthy control individuals. The breast cancer patients will be longitudinally followed for evaluation of the response to treatment in order to determine the prognostic and monitoring values of the assay. The high risk groups (fibrocystic breast disease and breast cancer families) are also to be followed in order to evaluate the diagnostic potential of the immune complex assay. The antigen-antibody complexes are also to be isolated from the serum and body fluids (pleural effusion) of breast cancer patients by molecular exclusion chromatography, protein A affinity chromatography and immunoadsorption using affinity media to the Fc domain of IgG. Further, the isolated immune complexes will be dissociated under the least denaturing conditions. The dissociated antigens are to be characterized biochemically and immunologically. Xenobiotic antisera will be raised with the dissociated antigen(s) and with isolated complexes as well as with the Raji cells containing surface-bound immune complexes injected into rabbits made tolerant to human IgG, and studied by their ability to recombine the dissociated antigen and to bind malignant breast tissues. In vitro antibody-dependent cell-mediated cytotoxicity, lymphocyte mitogenesis, PHA-induced lymphocyte blastogenesis and LAI assays are also to be performed to study the possible biological function of antibody-antigen complexes. Further, a radioimmunoassay will be developed which may be of use in early detection and monitoring of efficacy of therapy for breast cancer.

225. USE OF CELL MEMBRANES TO ASSESS PHENOTYPIC EXPRESSION

Ceriani, R. L., Peterson, J. A., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, *51st & Grove Sts., Oakland, California,* 94609, United States of America

The objective of this grant is to further define the normal mammary epithelial (ME) antigens, to study their altered expression in breast neoplasia and the reason for it, and finally to identify the effect of such alterations on ME cellular functions. Mammary epithelial (ME) antigens have been discovered by us both in humans (HME antigens) and in mouse (MME antigens). They are cell membrane components with cell-type specificity for normal mammary epithelial cells, which are also present in neoplastic ME cells. We will be using both models, human and mouse (complementing each other), to do the following: a) Continue to isolate and purify the ME antigens. This will be done using monoclonal antibodies of predefined specificity produced in hybrids between myeloma cells and spleen lymphocytes, the latter obtained from an animal immunized with mammary cell membrane components. Also, attempts will be made to separate ME antigens with the use of lectin affinity separation. b) Quantitate ME antigens with the aid of monoclonal antibodies in different stages and types of neoplasias, trying to correlate levels and possible changes with the neoplastic phenotype. The quantitation will be done by indirect immunofluorescence, radioimmunoassay, cytotoxicity and flow cytofluorometry. c) Study how ME antigen expression (presence, diminution, absence) affects function in ME cells, in such areas as cell replication, differentiation and expression of the neoplastic phenotype.

Further, we will continue our maintained effort at cell identification in the breast by extending our study of specific myoepithelial antigen(s) and fibrocyte antigen(s) of the breast that we have just defined.

BIBLIOGRAPHIC REFERENCES: Ceriani, R.L., Peterson, J.A., Blank, E., and Miller, S.: Tumor Markers: Impact and Prospects. In: Applied Methods in Oncology, Vol. 2 (Boelsma, E. and Rumke, Ph., eds) Elsevier, Amsterdam, p. 101, 1979. Sebesteny, A., Taylor-Papadimitriou, J., Ceriani, R.L., Millis, R., Schmitt, C., and Trevan, D.: Primary human breast carcinomas transplantable in the nude mouse. J. Nat. Cancer Inst. 63:1331, 1979.

226. PHYSICOCHEMICAL AND IMMUNOCHEMICAL ANALYSIS OF CANCER RELATED ANTIGENS

Franchimont, P., Zangerle, P. F., Hendrick, J. C., Osterrieth, Laboratorium de Radioimmunologie, Tour de Pathologie, *C.H.U. Bat. B23, Sart-Tilman, 4000 Liege, Belgium*

OBJECTIVES: A. Exocrine ectopic secretion: breast cancer, as neoplasia of other origin, can secrete milk products

such as casein and lactalbumin. Furthermore, a proteic tracer was identified in cystic fluid of mammary gland; this tracer is also detected in serum of patients with breast cancer. In the future, investigation will be directed to the immunological and physicochemical characterization of these mammary products. B. Isolation, purification and physicochemical and radioimmunochemical analyses of two new membrane antigens, one from bronchial neoplasm and the other from mammary carcinoma. C. Identification of antigenic groups of CEA which are organ cancer specific. D. Preparation and radioimmunoassays of viral antigens (P28, P47) involved in the mouse and monkey mammary carcinoma. Cross-reactions are presently detected by RIA, EIA, and immunofluorescence between these viral antigens and substances from cancer homogenate of patients with breast cancer. E. Isolation of immuncomplexes from blood of patients with breast and lung cancer. Characterization of the antigens involved in these immune complexes.

227. IMMUNE COMPLEXES IN PATIENTS WITH BREAST CANCER

Sulitzeanu, D., Hebrew University of Jerusalem, Hadassah Medical School, Lautenberg Center for General & Tumor Immunology, *P.O. Box 1172, Jerusalem 91000, Israel*

The purpose of this investigation is twofold: a. To follow the presence of immune complexes in patients with breast cancer, in order to determine whether any correlations exist between the level of the complexes and the course of the disease. b. To isolate immune complexes from the sera of patients and to analyze them in order to identify the antigens from which they are made.

Immune complexes are being assayed by a complement consumption test. They are purified by means of a rheumatoid factor adsorbent and are analyzed by SDS-PAGE.

228. HLA ANTIGENS AND SURVIVAL IN MAMMARY CARCINOMA PATIENTS

Pellegris, G., Muscolino, G., Bertario, L., Costa, A., Illeni, M. T., Leo, E., Saccozzi, R., Istituto Nazionale per lo Studio e la Cura dei Tumori, Immunohematology Laboratory, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: The aim of this work is to verify HLA typing as to whether immunogenetic factors may be suspected as playing any role in conditioning the survival of patients for over 8 years after surgical removal of mammary carcinoma.

APPROACH: Two groups of patients will be typed for HLA A,B and C antigen: one group showing relapse and/or metastasis more than 8 years after surgical removal of the tumor, the other being apparently free of tumor clinical signs. Each group will include 30 to 50 patients untreated by chemo- and/or hormonotherapy.

The lymphocytotoxic test will be used.

Controls will be healthy unrelated females, similar to the patients as regards age, and living in the same geographical area as the patients.

229. LOCALIZATION OF ANTI-CEA ANTIGEN AND ANTI-CDP ANTIBODIES IN BREAST CANCER

Cox, C. E., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina*, 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

230. TUMOR REJECTION ANTIGENS FROM TREATED CANCER CELLS

Silagi, S., Weintraub, S., HeaneyKieras, J., Cornell University, Medical Center, Cornell Medical College, Dept. of Obstetrics & Gynecology, *1300 York Ave., New York, New York*, 10021, United States of America

The purpose of this investigation is to solubilize, purify, isolate and characterize tumor rejection antigens from immunogenic mouse melanoma and mammary adenocarcinoma cells. The melanoma cells were rendered nontumorigenic and immunogenic by long-term culture in 1 microgram/ml of bromodeoxyuridine, and the mammary tumor cells by long-term culture in normal medium. Plasma membranes will be isolated by differential centrifugation from cells disrupted by nitrogen cavitation and fractionated on sucrose-Dextran and sucrose flotation gradients. Characteristics of the purified subcellular fractions will be monitored by radioimmune and biochemical assays. Immunogenicty will be tested both by in vivo tumor rejection assays and by in vitro assays worked out with whole cells. Soluble fractions will be prepared from the immunogenic fraction and purified by apropriate chromatographic methods. Immunochemical analysis of the membrane fractions will be carried out using antisera we have prepared. Biochemical studies on the glycosaminoglycans and proteoglycans of these cells will be made and correlated with their unusual biological properties. The ultimate aim of these studies is to use them as model systems forming a basis for safe immunization of melanoma and breast cancer patients after removal of their primary tumors.

231. CARCINOEMBRYONIC ANTIGEN IN BREAST CANCER PATIENTS

Zamcheck, N., Boston City Hospital, Gastrointestinal Research Laboratory, *818 Harrison Ave., Boston, Massachusetts*, 02118, United States of America

This is part of a broader project. A summary of this subproject is not available.

232. IMMUNE MECHANISMS INVOLVED IN TUMOR METASTASIS

Roholt, O. A., Kim, U., Fuji, H., Ghosh, S. K., Roswell Park Memorial Inst., Dept. of Immunology Research, *666 Elm St., Buffalo, New York*, 14263, United States of America

The summary for this project is under entry no. 146.

B. OTHER IMMUNOLOGY STUDIES

233. PRENEOPLASTIC POTENTIAL OF HUMAN BREAST LESIONS

Wellings, S. R., Faulkin, L. J., University of California, Davis Campus, School of Medicine, Dept. of Pathology, *Davis, California*, 95616, United States of America

Human breast biopsy tissues, no longer needed by the surgical pathologist, are transplanted into nude athymic mice in order to assess their growth potential. Normal, presumed preneoplastic, and neoplastic growth and differentiation are compared.

234. TRANSPLANTATION OF HUMAN MAMMARY DYSPLASIAS AND CARCINOMAS INTO NUDE MICE

Ozzello, L., Universite de Lausanne, Dept. of Pathology, *Pl. de la Cathedrale 4, Case Postale 611, 1000 Lausanne, Switzerland*

OBJECTIVE: To study the behavior of human mammary tissues transplanted into thymus-deficient nude mice.

APPROACH: Fresh tissues from surgical specimens and trypsinized cell cultures are grafted subcutaneously in adult BALB/c nu/nu mice as follows: 1) Various types of dysplastic lesions, mainly hyperplasias, to study their evolution under experimental conditions and their eventual neoplastic transformation; 2) solid carcinomas in an attempt to improve their rate of take; 3) serially transplantable tumors produced by subcutaneous injection of several human mammary carcinoma cell lines to study their metastatic potential and hormone dependency.

235. IMMUNE REACTIVITY AND MAMMARY NEOPLASIA

Heppner, G. H., Miller, F. R., Michigan Cancer Foundation, Dept. of Immunology, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

236. SPECIFIC IMMUNITY AND PROGNOSIS IN BREAST CANCER

Black, M. M., Zachrau, R. E., Leis, H. P., Isac, M. J., New York Medical College, Westchester Campus, School of Medicine, Dept. of Pathology, *Valhalla, New York*, 10595, United States of America

Individual cancer patients were tested repetitively, at increasing postoperative intervals by a leukocyte migration test (LMT) and a skin window (SW) procedure. One quarter of 48 patients demonstrated LMT reactivity 1-8, 9-14 and 15-24 months post-operatively while one-fifth of the patients were minimally responsive during each of these intervals. Among 21 patients showing minimal reactivity to autologous breast cancer 1-8 months postoperatively, approximately half were reactive 9-

14 and/or 15-24 months postoperatively. CMI to autologous breast cancer 9-24 months postoperatively was prognostically favorable regardless of the reactivity 1-8 months postoperatively.

The phenomenon of spontaneous variations in CMI to autologous breast cancer suggests the possibility of experimentally maintaining or inducing such reactivity. Spontaneous variations in LMT reactivity to autologous breast cancer also provide a device for evaluating the specificity of such reactivity by simultaneously testing other targets. Among individual patients, tested repetitively 9-50 months postoperatively, reactivity to autologous breast cancer was correlated with reactivity to gp 55 of R III MuMTV and to its (gp 55) glycopeptide fraction. No such correlation was found with fetuin, its glycopeptide, benign breast lesions or colon carcinoma.

Horizontal studies of simultaneous LMT reactivity to autologous breast cancer and selected targets provide important insights into the biological significance and specificity of such reactivity. Such information is pertinent to the development of immunotherapy and immunoprophylaxis and to the choice of patients for adjuvant chemotherapy.

237. LYMPHOCYTE FUNCTION IN BREAST CANCER

Harrison, R., Knight, S., Farrant, J., Clinical Research Centre, Oncology Unit, *Watford Rd., Harrow, Middlesex, United Kingdom*

OBJECTIVE: To investigate whether the study of two in vitro factors, (1) cell concentration and (2) harvest time, clarifies the relationship between the observed pattern of lymphocyte function and the clinical states of these.

APPROACH: Lymphocyte transformation can be induced by specific or nonspecific stimuli. This transformation may be measured by estimation of DNA synthesis. Lymphocyte transformation has been measured in patients with malignant disease but the correlation with the clinical stage of the disease has been variable. It has been established that a range of cell concentrations is important in assessing lymphocyte function (Knight S. and Farrant J., 1978, Journal of Immunological Methods 22 63-71). With the development of a simple harvester for 20 microlites of Terasaki cultures (O'Brien et al., 1979, Journal of immunological Methods, in press) and the relization that different harvest times are also required (Farrant J. and Knight S., in preparation) it is proposed that a re-examination of the relationship between lymphocyte function and disease is undertaken utilizing these new techniques. All new patients presenting with carcinoma of the breast and those presenting with recurrent disease will be considered for inclusion in the study. Initially 1-2 patients per week will be studied as dictated by available laboratory facilities.

PROGRESS: Patient entry to this study has now been limited to post-menopausal women to make the subsequent analysis of a single biological group possible. Control studies confirm the reproducibility of the method and are continuing to look at any natural variability.

238. IMMUNOLOGY OF BREAST CANCER (HUMAN, RABBITS)

Marcus, D., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Cellular Biology, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

This is part of a broader project. A summary of this subproject is not available.

239. IMMUNOLOGICAL STUDIES OF MICE BEARING PRIMARY MAMMARY TUMORS

Choppin, J. L., Benoist, H., Desplaces, A., Centre Rene Huguenin, Laboratoire, *5, rue Gaston Latouche, 92211 St. Cloud, France*

OBJECTIVES: To characterize the immune competence of C3H/Bi mice (MTV positive) before and during tumor growth. To search for enhancing and suppressor mechanisms involved in non-specific immune responses.

APPROACH: Controls of different ages and tumor-bearing mice are used. The immunologic tests include: 1) study of the immune response to SRBC, LPS and Oxazolone by means of RFC and PFC detection in spleen and lymph nodes. 2) antibody-dependent cytotoxicity (ADCC), 3) macrophage activities.

PROGRESS: Tumor growth exerts an enhancing effect on the primary responses to SRBC and LPS. Other studies are under way.

240. IMMUNOLOGIC STUDIES IN MOUSE AND HUMAN BREAST CANCER

Girardi, A. J., Dion, A. S., Holben, J. A., Inst. for Medical Research, Dept. of Virology & Immunology, *Copewood St., Camden, New Jersey,* 08103, United States of America

The long-term objective of the proposed research is to study the immunologic relationships between various mouse mammary tumor viruses by cross-protection tests in mice and to determine, through similar tests, possible relationships between mouse and human mammary tumors. We will test the vaccines prepared from mouse mammary tumors, purified MMTVs, and purified subviral components, gp55 (or gp50) and p28, in a relatively rapid screening system, using mice which ordinarily do not exhibit a high incidence of spontaneous disease but which are exquisitely sensitive to challenge by infectious MMTVs of the S and P types. These studies will serve as a guide for vaccination in mice which demonstrate high incidence of spontaneous disease and which require longer holding periods for analysis. Using the same strategy, we will study the relationship between the diseases in mice and mammary carcinoma in humans. Human tissues will be obtained from pre- and postmenopausal women demonstrating either infiltrating ductal carcinoma or lobular carcinoma in situ, as primary tissue or cell culture. In addition, extracts will be prepared from primary human tumors. In all studies appropriate controls will be used as immunogens including non-mammary tumors, benign lesions, non-B type viruses and subviral components of the latter. Regardless of vaccines used we will ask whether there is a reduction of or a delay in the appearance of: MMTV antigens which appear in milk of lactating mice prior to tumor formation; early or late mammary tumors which appear as a result of horizontally transmitted milk virus (early), or vertically transmitted endogenous virus (late); nodules (and tumors) in those strains of mice wherein 'L type disease' is active. We will ask whether the inhibition or delay which might be detected is virus 'subgroup' specific (e.g., S, L, P type) or virus and mouse strain specific (e.g., RIII, GR, C3H, A).

241. IMMUNOLOGY OF ANIMAL AND HUMAN TUMORS, WITH EMPHASIS ON VIRUS-INDUCED MAMMARY NEOPLASIA

Plata, E. J., Todaro, G. J., Schlom, J., Levine, P. H., Boot, L. M., Duenas, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Carcinogenesis Laboratory, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Natural antibodies and naturally expressed antigens associated with mammary tumor virus infection and neoplasia were detected and characterized in body fluids of mice as a model for the detection of similar markers in human tissues. Similar studies were started with herpes simplex viruses. These collaborative studies seek to clarify the role of these markers in the host's immune response to virus infection and the pathogenesis of malignancy.

242. CELL-MEDIATED IMMUNITY AND ASSAY FOR BREAST CANCER

Yonemoto, R. H., Chee, D. O., Nakamura, T., City of Hope Medical Center, *1500 E. Duarte Rd., Duarte, California,* 91010, United States of America

The goal for the proposal is to develop an assay of cell-mediated immunity in breast cancer with clinical applicability, i.e., prognosis, guide to therapy and diagnosis. The rationale for the proposal is: 1) Improvement of leukocyte adherence inhibition assay, 2) utilization of highly purified TAA which should result in greater specificity in the leukocyte adherence inhibition assay (LAI), 3) parallel use of normal tissue antigen from same patient as control, 4) the use of sensitized frozen lymphocyte (allogeneic) because of easy accessibility of large quantities, access to repetitive determinations at convenience, and easy application of blocking assay, 5) in addition, basic understanding of the mode of action of LAI, i.e., cellular participation, lymphokine study, interplay of blocking factor, which will lead to an improved assay. To accomplish this goal, studies will be performed in two categories of patients.

A vertical study (patients to be entered during first two years) includes patients with benign and malignant disease, to seek comparative efficiency of LAI utilizing fresh PBL vs. frozen lymphocytes. A horizontal study (1st, 2nd and 3rd year) will evaluate patients at 1, 3 and more than 5 years post-mastectomy with no evidence of disease, as well as all patients at point of recurrent disease. All patients will undergo LAI and blocking assays at 3 month intervals as follow-up to therapy.

An improved CMI assay to monitor breast cancer patients will materialize at the end of 3 years.

BIBLIOGRAPHIC REFERENCES: Tanaka, Fumitaka, Yonemoto, Robert H., and Waldman, Stephen R. Blocking Factors in Sera of Breast Cancer Patients. Cancer, 43 (in press), 1979.

243. IMMUNOGENETIC STUDY OF BREAST CANCER AND LEUKEMIA

Boot, L. M., Nederlands Kankerinstituut, *106-108 Sarphatistraat, Amsterdam, Netherlands*

The Contractor will conduct immunogenetic studies on the hereditary transmission of the mammary tumor virus in the GR strain in which the genetic influence associated with breast cancer is similar to that observed in humans.

244. IMMUNOLOGICAL EVALUATION OF PATIENTS WITH RAPIDLY PROGRESSING BREAST CANCER

Mourali, N., Epstein, R., Tabbane, F., Belhassen, S., El Meddeb, A., Salah Azaiz Inst., Laboratory of Immunology, *Blvd. du 9 Avril, Bab Saadoun, Tunis, Tunisia*

OBJECTIVE: To determine the general immunological status of patients with rapidly progressing breast cancer (PEV) using a battery of in vitro and in vivo immunological assays designed to measure the immune competency of the host. It is hoped that these procedures can be shown to be of value clinically to monitor the patient's progress during a treatment protocol consisting of combination chemotherapy and either radiotherapy or surgery.

METHODS: PEV positive patients with no evidence of clinical metastasis will undergo 18 cycles of chemotherapy. After the third cycle they will be randomized between radiotherapy and surgery. At monthly intervals commencing before any treatment is initiated, peripheral blood samples will be obtained from each patient. Lymphocytes will be purified and nonspecific reactivity to PHA, ConA and PWM will be determined using a tritiated thymidine uptake assay. Crude antigen will be prepared from autologous tumor samples and patients' reactivity toward their tumor will be analyzed by thymidine incorporation. Other in vitro CMI assays to be employed will be MLC, migration inhibition, PHA induced cytotoxicity, antibody dependent cellular cytotoxocity and a new assay denoted as SINEC (specifically induced nonspecifically expressed cytotoxicity). In SINEC lymphocytes are specifically activated by tumor antigen and will nonspecifically express their cytotoxic capability by lysing unrelated targets. Additional immune parameters to be used will include skin testing and T cell rosetting.

245. IMMUNOLOGICAL STATUS AND ANTI-TUMOR ACTIVITY OF PATIENTS WITH MAMMARY CARCINOMA

Coombs, R. R., Eremin, O. F., University of Cambridge, New Addenbrooke's Hospital, Dept. of Pathology, *Hills Rd., Cambridge CB2 2QQ, England, United Kingdom*

OBJECTIVE: To establish whether there is, in patients with mammary carcinoma and other solid tumors, a tumor-specific immune response to the tumor.

APPROACH: Tumor-specific lymphocyte reactivity is monitored in a sequential manner in women with mammary carcinoma in a one-way mixed tumor lymphocyte interaction (autochthonous tumor cells). The expression of this reactivity will be correlated with the clinical course of the disease. The possible role of suppressive factors in the tumor cell mass itself, particularly host reactive cells (suppressor lymphocytes, macrophages) inhibiting a tumor-specific allergic response, will similarly be ascertained. The infiltrating host reactive cells will be isolated from the tumor cell mass and their properties investigated.

PROGRESS: To date no tumor-specific allergic response has been detected at the time of operation in patients with mammary carcinoma. Preliminary findings suggest that, following surgery, some patients appear to show a tumor-specific allergic response.

246. IMMUNOLOGIC RESPONSE OF BREAST CANCER PATIENTS TO BREAST CANCER EXTRACTS

DellaPorta, G., Canevari, S., Fossati, G., Miotti, S., Orefice, S., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To study by in vivo and in vitro tests the antigenicity and specificity of soluble extracts obtained from pools of fresh breast carcinoma specimens and from a breast cancer cell line (MCF-7).

APPROACH: Soluble crude material obtained by 3 M KCl method will be further purified by different immunochemical and biochemical procedures. The immunogenicity and specificity of various preparations will be monitored by delayed cutaneous hypersensitivity reaction (DCHR) and by two in vitro tests: the leukocyte adherence inhibition and the agarose leukocyte migration inhibition.

PROGRESS: 3 M KCl soluble extracts from two pools of breast cancer specimens gave positive response by DCHR in 69% (first pool) and in 90% (second pool) of breast cancer patients, whereas soluble material from a normal breast was positive in 19% of breast cancer patients.

247. NATURAL AND ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY IN MAMMARY CARCINOMA PATIENTS

Bakacs, T., Garam, T., Svastits, E., Ringwald, G., National Inst. of Oncology, Tumor Immunology Section, *P.O. Box 21, H1525 Budapest, Hungary*

OBJECTIVE: Natural cell-mediated cytotoxicity (NCMC) and antibody dependent cytotoxicity (ADCC) may be envisiged as a second line of defense to specific T cell mediated disease related cytotoxicity. There are, in fact, several reports which claim NCMC and ADCC decline as malignant disease progresses. Whether this is a result of or the cause of the disease is a matter for discussion, but these reactions could be used to monitor general immune responsiveness in malignant patients.

NCMC and ADCC reactions measured in the classical way did not show the sensitivity expected. It was discovered that lymphocyte cytotoxic activity functions much like enzymes do. In other words cytotoxic activity of a constant number of lymphocytes can be increased until a plateau is reached by increasing the target cell number. The maximal activity measured this way we called cytotoxic capacity. Obviously this capacity is underappreciated if tested in the classical way. Therefore we have started to measure NCMC and ADCC activities of mammary carcinoma patients in the cytotoxic capacity test. We collect blood and test lymphocyte cytotoxic capacity before, during and after treatment, and seek a correlation between activities measured and treatment. Experiments have already started and more than 40 patients have been tested. We will terminate this project at the end of the year.

APPROACH: Peripheral blood of mammary tumor patients is collected in heparinized tubes. Mononuclear cells are separated according to Boyum. Macrophages are removed according to Jondal et al. Thereafter, lymphocytes are tested against K-562 myeloid leukemic cell line (NCMC) and against sensitized human Rh positive red blod cells (ADCC) in a 51Cr-release test.

248. IMMUNOLOGICAL STATUS OF BREAST CANCER

Desplaces, A., Brunet, M., Berlie, J. G., Gest, J., Bourguignat, A., Oglobine, J., DeRicqles, D., Saracino, R. T., Centre Rene Huguenin, Laboratoire, *5, rue Gaston Latouche, 92211 St. Cloud, France*

OBJECTIVE: The aim of the present work is to utilize examinations performed for several years in order to determine the immunological status of the breast cancer patients.

APPROACH: This research will: 1. Study the homogeneity of test responses and dosages with respect to the elaboration of standards. 2. Reveal an eventual relationship between the patient's profile, disease and pre-treatment examinations. 3. Evaluate these results in course of time in correlation with therapeutic methods and cancer history.

249. PROTECTION OF MICE FROM DEVELOPING MAMMARY TUMORS BY VACCINATION WITH MTV PROTEINS

Michalides, R. J., Boot, L. M., Ropcke, G., Nederlands Kankerinstituut, Div. of Virology, *108 Sarphatistraat, Amsterdam, Netherlands*

OBJECTIVE: To protect mice from developing mammary tumors, induced by either MTV or hormones, by vaccination with formalin-treated MTV.

APPROACH: Newborn mice at the age of 3-4 weeks are vaccinated with 10 micrograms of formalin-treated MTV and subsequently after one week either infected with MTV, stimulated with hormones by implanting a hypophyseal isograft or not treated at all. The mammary tumor incidence is registered. In addition, blood is taken from these mice once every month by retro-orbital puncture; the presence of antibodies directed against MTV is measured using the MTV-radioimmunoprecipitation (RIP) assay.

PROGRESS: Strains of mice with a low mammary tumor frequency, such as 020 and C57BL, do not possess antibodies directed against MTV. While only some mice of strains with intermediate mammary tumor frequency, such as C3Hf and BALB/c, do possess Ab against MTV, the majority of mice of high mammary tumor strains, such as GR and C3H, possess Ab against MTV. The development of humoral immune response directed against MTV precedes the mammary tumor development. Treating animals with prolactin to increase mammary tumor frequency also increased the proportion of animals carrying Ab against MTV.

250. HEMATOLOGIC AND IMMUNOLOGIC STUDIES IN BREAST CANCER PATIENTS

Nemoto, T., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

251. OBSERVATIONS ON THE IMMUNOLOGICAL STATUS OF RATS WITH EXPERIMENTAL BREAST CANCER

Ciocca, D. R., Moviglia, G., Santolays, R. C., Instituto de Histologia y Embriologia, *C.C. 56, 5500 Mendoza, Argentina*

Rats with pituitary implants underneath the renal capsule are a good experimental model with which to study the immunobiology of breast cancer, due to the fact that hormonal imbalance is considered to be responsible for the tumorigenesis in these cases. Besides, rats have long survival which allows for the evaluation of the effect of aging. Regional and distant lymph nodes in relation to the site of primary tumor growth have been studied in this experimental model, using light microscopy and electron microscopy. Both types of lymph nodes (regional and distant) have been shown to have the same reaction, that is a depression of sector T and hyperstimulation of sector B.

In a second phase, ultrastructural studies of the thymus and spleen are being performed. At a later stage, functional studies of sector B will be undertaken.

252. IMMUNE REACTIVITY AND MAMMARY NEOPLASIA

Heppner, G. H., Medina, D., Miller, F., Brown University, Roger Williams General Hospital, *825 Chalkstone Ave., Box 1883, Providence, Rhode Island*, 02908, United States of America

Transplantable lines of preneoplastic, hyperplastic alveolar nodules and ductal hyperplasias will be used to study the nature of immune reactivity to an emerging tumor. Mice bearing these lines will be treated with chemical carcinogens which have been shown capable of influencing the conversion of preneoplastic lesions to frank neoplasia. The immune responses directed against these lines and their associated tumors will be assessed by microcytotoxicity, macrophage migration inhibition, lymphocyte blastogenesis, 51Cr cytotoxicity, Winn, and transplantation resistance assays. Emphasis will be placed on characterization of the immune reactivity within the mamary fat pad and lesion mass. In addition, the ability of immune cells to stimulate tumor cell survival will be investigated.

253. ROLE OF TUMOR MACROPHAGES IN MAMMARY TUMOR METASTASIS

Heppner, G. H., Loveless, S. E., Michigan Cancer Foundation, Dept. of Immunology, *110 E. Warren St., Detroit, Michigan*, 48201, United States of America

The overall objective of the research project is to determine the role of tumor-associated macrophages in the metastasis of different subpopulations of mammary tumors which all arose from a single autochthonous murine mammary tumor. The specific aims of this project are to: 1) Characterize the tumor-associated macrophages obtained during the growth of different tumor subpopulations derived from a single mouse mammary tumor in terms of number, surface receptors, density and enzyme markers. 2) Describe the effect of depletion of tumor-associated macrophages on the subsequent growth and metastasis of tumor subpopulations. 3) Determine whether tumor-associated macrophages isolated from different subpopulations of mammary tumors exert an enhancing or inhibitory effect on tumor survival and ascertain its specificity.

To approach this proposed research, tumor-associated macrophages will be characterized with respect to number, as determined by the number of adherent, Fc and nonspecific esterase positive, trypsin resistant cells contained in an enzymatically disaggregated tumor cell suspension which are capable of immune phagocytosis. Density differences within the population of macrophages will also be examined using a discontinuous ficoll gradient. Macrophage-depleted tumor cell suspensions will be: 1) plated in culture and in vitro doubling time calculated and 2) reimplanted into syngeneic hosts and monitored for latency period, growth rate and metastatic potential. Finally, macrophages isolated from mammary tumors will be utilized in various in vitro assays designed to examine their effect on tumor target cells and also their ability to phagocytize.

254. FACTORS NEGATING RESPONSE TO MAMMARY ADENOCARCINOMA

Lucas, Z. J., Stanford University, School of Medicine, Dept. of Surgery, *Stanford, California*, 94305, United States of America

We have found that the syngeneic hosts' immune responses in the spleen to the 13762A mammary adenocarcinoma are cyclic. An initial cytolytic cell response, peaking approximately 7 days after tumor inoculation, declines between days 10-14, only to peak again on or about days 18-20. Cytolytic cells in both lytic phases are T-lymphocytes. The period of depressed cytotoxic cell responses is accompanied by increased humoral blocking factors and increased numbers of splenic suppressor cells.

The blocking factors are heterogeneous, consisting of tumor antigen, antigen-antibody complexes and an IgG factor appearing to be an anti-receptor site antibody. Only the latter decreases during the second wave of splenic cytotoxic cells. Investigations of the stimuli eliciting the blocking IgG and suppressor cells are proceeding.

BIBLIOGRAPHIC REFERENCES: Huber, S., Walker, S. M., and Lucas, Z. J. Microcytotoxicity assay for cell-mediated immunity enumerating residual target cell number by 86 Rb. J. Immunol. Methods 24:287, 1978. Huber, S. and Lucas, Z. J. A quantitative assay for tumor antigen based on inhibition of cytotoxicity of sensitized lymphocytes. Intl. J. Cancer 23:224, 1979.

IV. BROAD STUDIES OF BREAST CANCER

255. GORDON RESEARCH CONFERENCE ON MAMMARY GLAND BIOLOGY

Hilf, R., University of Rochester, School of Medicine & Dentistry, Dept. of Biochemistry, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

The fifth biennial Gordon Research Conference on Mammary Gland Biology will be held 18-22 June, 1979, at Holderness School, Plymouth, N.H. These conferences bring together scientists of many disciplines, all of whom share a major research interest in the mammary gland and whose research interests are broadly diversified. Conferences have been held in alternating years since 1971 and the attendance for these conferences has been: 1971, 50; 1973, 81; 1975, 76; and 1977, 123. It

should be noted that the large increase in attendance from 1975 to 1977 probably reflects the continued interest in the need for a multi-disciplinary approach in mammary gland research, and a strong desire of the scientific community to relate this information to the problem of breast cancer. Indeed, the inclusion of sessions devoted to exciting new developments in the field of mammary cancer has fostered the enthusiastic interactions of the attendees. On the basis of the interest expressed to date, we anticipate an attendance of 100 to 125 participants in 1979.

256. BREAST CANCER STUDY (HUMAN)

Foster, R. S., University of Vermont & State Agricultural College, School of Medicine, Dept. of Surgery, *85 S. Prospect St., Burlington, Vermont*, 05401, United States of America

This is part of a broader project. A summary of this subproject is not available.

257. CELL BIOLOGY OF MAMMARY GLAND NEOPLASIA

Slemmer, G. L., University of British Columbia, Dept. of Pathology, *2075 Wesbrook Pl., Vancouver, British Columbia*, V6T 1W5, Canada

A program to study the cell biology and immunology of normal and neoplastic mammary glands has been developed. Using transplantation methods with the aid of histocompatibility markers, mammary parenchyma or parenchymal-stromal combinations composed of cells of different genotypes will be studied to analyze cell types and cell interactions during neoplastic progression and host responses to cryptic antigenic cells.

Three separate cell populations comprising normal breast give rise to 3 classes of neoplasia. Normal cell associations are required by neoplastic cells of some types during neoplastic progression and metastasis. The correlation of cell type of origin, cellular composition, and interdependence of cells with histopathology, growth, and metastatic characteristics will be studied.

The afferent limb of the cytotoxic cellular immune response is blocked by anatomical or physiological factors in some epithelial, endocrine, and neoplastic tissues. Persistent antigenic cells induce specific suppressor responses simulating normal self tolerance. Qualitatively different immune responses susceptible or resistant to this suppression exist. Suppressor responses permit allografting without general immunodepression. Immune responses resistant to suppression cause regression of established neoplasms. Methods for induction of natural suppression of allograft immunity and for induction of effective immunity to various types and stages of mammary neoplasia will be studied.

258. BIOLOGY OF MAMMARY NEOPLASIA

Nandi, S., Bern, H. A., Blair, P. B., DeOme, K. B., Pitelka, D. R., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California*, 94720, United States of America

The program focusses on factors involved in the development of benign and malignant mammary and cervicovaginal lesions in mice. The objects of proposed studies include: The origin of inapparent transformed cells in normal mouse mammary tissues and the nature of cellular and host factors leading to their emergence as overt lesions. The presence of components of the MTV genome in DNA of mouse germline cells. Chemical carcinogen-induced transformation of rat mammary cells in vitro. Permeability and transport parameters of mammary tumor cell monolayers in culture on floating collagen gels. Influence of perinatal plus later treatment with steroid hormones on development of mammary and cervicovaginal tumors in mice and the sites of hormone action. Distortion of subsequent immune reactivity in mice given perinatal steroid treatment and the relation between immunologic changes and risk of neoplasia. Long-term effects on survival of mice after single-dose treatment with chemotherapeutic drugs. The role and fate of cellular factors responsible for epithelial cohesiveness in experimental invasion of other tissues by malignant mammary tumors. The influence of epithelial cell shape on hormone-induced secretory differentiation of mammary cells in culture.

259. PROTOTYPE NETWORK DEMONSTRATION PROJECT IN BREAST CANCER

Whitney, L. W., Wilmington Medical Center, *501 W. 14th St., Wilmington, Delaware*, 19899, United States of America

The Contractor shall commence with the implementation stage of his prototype network demonstration project in breast cancer.

260. PROTOTYPE NETWORK DEMONSTRATION PROJECT IN BREAST CANCER

Carter, A. C., State University of New York, Downstate Medical Center, School of Medicine, Dept. of Medicine, *450 Clarkson Ave., Brooklyn, New York*, 11203, United States of America

The Contractor shall commence with the implementation stage of his prototype network demonstration project in breast cancer.

261. CLINICAL AND EXPERIMENTAL BREAST CANCER RESEARCH

Dao, T. L., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

The objectives of this program/project can be summarized as follows: 1. To undertake a multidisciplinary study to elucidate the mechanisms of carcinogenesis of the mammary gland in the rat. 2. To study the biochemical mechanism of the action of three major hormones, prolactin, estrogen and progesterone, on the mammary gland during normal physiological processes and during the process of carcinogenesis. 3. To elucidate the morphogenetic and host factors which contribute to the development of breast cancer. 4. To carry out biochemical studies to elucidate the nature of hormone dependency of breast cancer in humans. 5. To study serum and tissue sialyltransferase in patients with breast cancer. 6. To study the hormonal control of lactose synthetase in mammary gland and mammary tumors. 7. To study estrogen metabolism in women with breast cancer and investigate the possible sources and disposition of extragonadal estrogens.

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